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THE BOTANICAL REVIEW

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LICHENS
THEIR BIOLOGICAL AND ECONOMIC SIGNIFICANCE[1]

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[1] Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, No. 215.

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PREFACE

The principal purpose of this paper is to consider the economic uses of lichens. Because the general student may have had little association with lichenology, it is felt, however, that a preliminary discussion of the more important biological aspects might be helpful.

Lichenology is a large and specialized field which this paper cannot hope to encompass. Therefore, the material presented in the first part is, necessarily, of a very limited nature. It consists of a compilation of papers dealing with the morphology, symbiosis, reproduction, growth, physiology, taxonomy, distribution, ecology and origin of lichens, with special emphasis on publications since 1921. Because it has been possible to examine only part of the most recent literature, the data of A. L. Smith's book "Lichens" have been chosen as the most convenient, for it reviews all previous work in great detail and includes a full bibliography, notably on the older works. Nevertheless, in some cases it has been found essential to refer to publications prior to 1921. This is true, chiefly, of the latter part of the paper, for the endeavor here is to bring together all information available pertaining to economic and industrial uses of these plants.

I am indebted to many, particularly Dr. P. F. Scholander, for valued suggestions and criticisms. To Dr. K. V. Thimann, Dr. D.

H. Linder, Dr. W. H. Weston, Dr. P. C. Mangelsdorf, Dr. A. H. Hill, who have been kind enough to read part or all of this manuscript, I express my very deep appreciation, but at the same time absolve them all from responsibility for errors that may appear.

BIOLOGY

Lichens are plants consisting of light-colored hyphal filaments and cells or groups of cells of a green, blue-green, or more rarely brownish or reddish color. The green bodies and hyphal filaments are in close relationship with each other and contribute to the formation of a quite constant structure, the thallus, which in form varies according to habitat. Lichens are found on a wide variety of substrata, from the High Arctic to the Tropics and from mountain top to seashore, contributing richly, in some cases, to the flora of a region. The total number of known species is over 15,500, comprising 60 families and over 400 genera (232).

Morphology

Macroscopic Structure. Though the thallus may range in shape from leaf-like to filamentous or even to a powder-like entity, it is generally classified into one of three types—foliose, fruticose or crustose. The fruticose type may be somewhat specialized, so that the erect thallus is referred to as the podetia[2], with superficial outgrowths known as squamules[3]. In size, the lichen thallus varies from the obvious to the microscopic. The dorsal surface may be smooth to pustulate and may bear outgrowths such as cilia, isidia[4], branchlets, or cephalodia[5]; the last occasionally occurring below the thallus surface.

The spermagonia[6] or male reproductive bodies which bring about fertilization resulting in formation of the fruit or apothecia, and the soredia[7] are also usually to be seen on the upper surface, while the lower surface may have cyphellae or breathing pores as well as cilia and the coarser rhizinae. The hypothallus is the first growth of the thallus before differentiation; it persists as a dark, ring-like, marginal outgrowth common to some crustaceous forms; its function is not very clear.

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[2] Podetia: alga-bearing branched or unbranched stalks, rising from the primary or horizontal thallus in certain lichens and bearing the ascocarp.

[3] Squamule: a small scale.

[4] Isidia: coral-like outgrowths on thalli in foliose and fruticose forms.

[5] Cephalodia: small regular or irregular outgrowths appearing on the surface of a few lichen thalli, containing algal cells and hyphae, usually enclosed finally in plectenchymatous cortex (63).

[6] Spermagonia: it appears to the writer that this customary spelling is incorrect and should be spermatogonia.

[7] Soredia: the accepted spelling of this word is incorrect; should be soridia.

Microscopic Structure.

a. Foliose Lichens. The green bodies and the colorless hyphal filaments may be interspersed through the entire thallus ("homoeomerous") or they may be arranged in distinct layers ("heteromerous") so as to present a false parenchyma-like appearance. In the heteromerous type of thallus there is an upper cortex consisting of more or less vertical hyphae without intercellular spaces; its surface may or may not be externally limited by an epidermis-like layer of hyphae (dermis). Beneath this there is an area of interwoven hyphae in which the intermingled green cells are enclosed. These cells were once thought to be the reproductive parts of the plants and were described as "gonidia" if green, or "gonimia" if blue-green. Because of this the green zone is often referred to as the "gonidial layer" or, more recently, as the "algal layer". The third layer is the medulla composed of loosely interwoven hyphae beneath which there is a compacted layer of hyphae, the lower cortex, and from this the rhizinae grow out. This is, in general, the microscopic structure of the foliose lichen thallus; to a certain extent, this is the structure in crustose and fruticose lichen thalli also, though, owing to their habit of growth, variation and intergradation occur.

b. Crustose Lichens. Crustose lichens growing over rock surfaces are referred to as "epilithic" or, if penetrating their substratum, as "endolithic" forms; both together come under the grouping of "saxicolous" lichens. If the substratum is wood or bark, the thallus may be immersed ("hypophloeodal") or growing only on the surface ("epiphloeodal"), and in either case it is known as a "corticolous" lichen. Because of their peculiar type of habitat, the thallus is considerably modified and may range from the homoeomerous to the heteromerous type of structure.

c. Fruticose Lichens. The fruticose lichen thallus, because of its more or less cylindrical form, appears somewhat more specialized. It consists of an outer pseudocortex of densely interwoven hyphae extending either in the direction of the axis or at right angles to it. Within this is the algal layer which may be evenly disposed along the cylinder or in patches; it may, in turn, have a medullary layer filling the remainder of the space, thus forming a solid core; this is frequently poorly developed or lacking.

Another and less evident definition of the different types of thalli is "endogenous" and "exogenous". The former includes a certain number of the homoeomerous forms in which the green cells are thought of as the predominating and determining factor forming the thallus; in the latter, the hyphae take the lead in thalline development. This interpretation is based on the fact that the algal layer sometimes is highly gelatinous, thus dominating the thallus form, though there is a certain amount of gelatinization present in all lichens.

History

Before considering the disposition of the green layer and its relationship to the hyphal filaments in lichens, it is of interest to note some of the concepts that have evolved from investigations of this unusual plant combination. Lichens were first segregated as such by Tourneforte in 1694. Early lichenologists assumed for a long time that these plants were perfect and complete in themselves and not phases of algae, fungi, mosses and liverworts. According to their knowledge, it was held that the variously colored bodies in lichens arose probably from the hyphae, though some workers noted the similarity between the green and blue-green cells in these plants and certain free-living algae. Schwendener became convinced that it was more than a similarity, and on the basis of his anatomical studies stated that lichens were composed of two distinct portions, fungal and algal. The "compound organism" concept gradually became accepted and with it arose an explanation for the association as a case of symbiosis. Symbiosis with certain modifications is generally accepted, though the idea of autonomy still persists, as may be noted in the works of Elfving[8]. The problem of whether the fungal or algal symbiont ever becomes free in nature and is capable of living free during its whole life-period outside the symbiotic association is one which has been closely questioned. The autonomous conception may be anathema to most biologists, but it deserves certain considerations that may lead to a clearer understanding of these unusual plant combinations.

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[8] Bibliography reference numbers are given in the text only when there is more than one citation for an author.

Lichen Components. Every individual of a given lichen species, as far as is known, contains the same alga and fungus, giving to the whole plant a relatively constant form and structure. Occasionally, an abnormal development of the thallus, or cephalodia, contains an algal symbiont foreign to that found in other portions of the thallus. In the majority of lichens, Ascomycetes predominate as the fungal component; in three tropical genera, these are Basidiomycetes, while in a few others, Fungi Imperfecti are present. Thus, a primitive lichen, *Botrydina vulgaris,* has been described in which the fungal component appears to be a Hyphomycete of the Fungi Imperfecti (1). Mattirolo devised the term "Hymenolichen" for those lichens characterized by the association of an alga and a Basidiomycete; while the Ascolichen refers to the combination of ascomycetous fungi and algae.

The algal component may appear as a filamentous or non-filamentous type in the lichen thallus and has been referred, according to Schwendener's conception, to free-living forms. Thus, it may belong to the Chlorophyceae, most commonly to the genus *Cystococcus, Pleurococcus* or *Trentepohlia,* or to the Myxophyceae, most commonly to the genus *Nostoc, Gloeocapsa* or *Rivularia.*

Recent Investigations. Investigations on the type of gonidia present in the lichen thallus have contributed much concerning this association. Paulson (160–162) found that the gonidia of most lichens belong to a species of *Chlorella,* the cells of which do not divide vegetatively but reproduce by sporulation within the algal mother cells, much as in free *Chlorella* cells.

These views do not coincide with those of Warén and others interested in the behavior of the lichen thallus in culture media. Warén cultivated bright-green gonidia from 21 species of lichens, finding that they grow best on amino acid media. He classified the gonidia of most of our common lichens under the genus *Cystococcus* consisting of section *Eucystococcus* (*Protococcus*), in which there is vegetative division, and *Eleuterococcus* which shows autospore formation. Moreover, the gonidia from the different species show differences of form and color in the culture colonies, which led him to think that each lichen species had its own particular algal, perhaps not free-living symbiont. In this case it is interesting to note that the algal symbiont of many lichens, as well as the fungal component, has been successfully cultured. Bioret, however, points out that in organisms so polymorphic as green algae, it is impossible to determine such specific or even racial aberrations. Warén further found that the gonidia in *Xanthoria parietina* (L.) Th. Fr. [= *Teloschistes parietinus* (L.) Norm.] from Finland differed from those of the same species collected in Holland.

Mameli (136), in her chemical studies of blue-green lichens, decided that in the lichen thallus the algal constituents suffered no important change except occasionally in increased size of the cells.

Variations in gonidia of lichens have been found by Linkola who cultivated *Nostoc* gonidia from eight different species of *Peltigera*. In *Peltigera malacea*[9], he thought that the variation may indicate some specific difference or only some particular physiological race. Chodat, in his studies of *Pleurococcus* gonidia of different lichen species, recognized them as being different from *Cystococcus* gonidia. During vegetative division the cells formed are easily confused with *Stichococcus*, explaining the view held by some lichenologists that at times gonidia of *Pleurococcus* type are modified by action of hyphae to *Stichococcus* forms.

Lichens have also been described in which the fungus and alga (217) were claimed to be associated with autotrophic bacteria (purple bacteria), but it has been proved conclusively that this is not so (207, 113); the purple color resulted from the presence of a lichenic acid secreted by the hyphae of the fungus. Cengia-Sambo (22) reports *Azotobacter* species in association with the fungal and algal component of a lichen.

Review of Theories. Schwendener, in presenting his hypothesis of the dual nature of lichens, believed this mutual relationship to be a state of parasitism of the fungus on the alga. If symbiosis is used in its generally accepted meaning as a harmless but intimate cellular association between at least two individuals belonging to different species, then it was Reinke who established the theory of lichen symbiosis. His term "consortium" was defined as two partners living peaceably together and working for the common good of the whole complex organism. Elenkin (50) found that destruction of algal cells goes on to a large extent at the lower side of the algal zone, his "necral zone". On this basis he proposed the theory of "endosaprophytism", indicating that the fungus ultimately devours the older algae. Smith (196), though accepting Elenkin's findings, preferred the symbiosis theory on the basis that such destruction of algal cells represents the normal wear and tear, which, in lichens, is compensated for by new growth from the gonidial region.

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[9] Names of authorities will be omitted except in discussing synonymy.

This has been well verified by studies on the relation between alga and fungus to determine the frequency of penetration of algal cells (163). There was not a single instance of penetration of algal partner by fungal hyphae in several common British species examined. They noted two forms of contact between the hypha and gonidium. In one, the gonidium was surrounded by loosely applied hyphae; in the other, the hypha was modified in shape so as to present a considerable surface to the gonidium. Many of the gonidia were dead, apparently because of "crowding together and lack of air", and the contents of such cells were absorbed by fungus hyphae, often without penetration of the wall. The relation between alga and fungus presents problems somewhat analogous to the mycorrhiza relationship in the Orchidaceae, which led Paulson and Hasting to suggest that each lichen may have to be taken on its own merits.

In contrast, Tobler (211) has recorded some interesting observations on this questionable relationship. He noted that at times the gonidia perished in the grip of hyphae where conditions favored the latter; but he also found examples of gonidia, perhaps favored by conditions of moisture, increasing enormously at the expense of the hyphae. He concludes that for the most part in the lichen thallus there is a constant balance of conditions between the two symbionts.

Bachmann (9), in the study of fungus galls of Cladoniaceae, affirms as a true symbiotic relationship what had been considered a form of parasitism. Mameli-Calvino (136) found occasional dead algae in the thallus, chiefly in the deeper layers where light is scarce.

Smith (196) further described lichens as being the most complete case of symbiosis. The balance is a delicate one but there is experimental proof for its existence. The Graphidaceae are cited as an example of the striking degree to which the symbionts are mutually dependent. Until the algae and fungi come into contact, they lead a meager existence, but after the relationship has been established, each partner is seen to take on new vigor. Miss Smith points out that this true symbiosis or mutualism by which the fungus has acquired so much vigor, has given rise to a whole new class of plants with well defined peculiarities of structure and of cell products. The algal component has been less affected, but has gained in endurance against time, unfavorable conditions and, in some cases, in actual size of cells.

Nienburg and others suggested the term "helotism" or slavery, since the algae were apparently held by the intermingling hyphae of the fungi. Fry (72) considered this term untenable, maintaining that parasitism prevails though unaccompanied by any morphological distinction. Danilov speaks of a disease of the alga caused by the fungus, while Moreau (148) looked upon the lichen fungus as a gall structure caused by the exciting action of the alga. McWhorter advances as proof of the parasitic nature of lichen hyphae, the cases he observed in which mosses were destroyed by lichens. This was partly due to parasitism and partly due to smothering. This confirms Fink's (59) definition that "a lichen is a fungus which lives during all or a part of its life in parasitic relation with an algal host and also sustains relation with an organic and inorganic substratum". Martin compares a *Gymnosporangium* gall of a cedar tree to the lichen thallus. Both are specialized and characteristic structures. Both are the result of two organisms, host and parasite reacting upon each other. The only difference is in size. In the cedar apple the host is larger than the parasite; in the lichen thallus the parasite is larger.

Darbishire (36) concluded that the components of lichens cannot be treated separately, for as lichens they exist in a new, physiological tradition. The lichen has shaken off the habits, morphologically and structurally, of its parasitic or saprophytic ancestors. The total result is evident in the remarkable convergence in type of species, not necessarily related but growing under similar conditions.

Schwendener, while working on the histology of the lichen thallus, noted the great similarity in the structure of the algal cells and fungal filaments. The algal cells, but for the presence of chlorophyll, had much in common with the colorless hyphae. More recently, Elfving, after careful study, suggested a genetic relationship in that gonidial cells, generally considered to be algal, are derived from colorless hyphae which are regarded as fungi. Strato observed *Peltigera canina* in nature and in transplanted cultures, in relation to growth and regeneration of the thallus. The results obtained by this author indicated that the algal host cells did not originate from the hyphae but were carried into regenerated areas by the developing hyphae, thus contradicting the views held by Minks and later affirmed by Elfving.

Reproduction

Asexual. Lichen reproduction may be sexual or asexual. In the latter, minute bunches of tangled hyphae with algal cells occur as soredia on the surface of the thallus and when blown loose by wind and deposited on the proper substratum, are capable of developing into thalli.

Herrmann has studied the development of soredia in Cladoniaceae and found that on a suitable substratum and under favorable conditions of moisture, light and temperature, the soredia develop into homogeneous thalli. First, the elongated, grasping or bridging hyphae grow out of the soredia, later followed by shorter ensnaring hyphae. This makes for a close alliance between the symbionts through entwining and enmeshing of the hyphae. The gonidia (*i.e.,* algae) divide, and at the same time intensification of color is brought about, perhaps, in response to stimulus of the lichen fungus. Division of free gonidia proceeds with a great reduction in the dimensions of the daughter gonidia which eventually die, as do the older enmeshed gonidia. The union of margins of neighboring soredial complexes by branching hyphae, and their fusion with one another, produces a morphological entity which is capable of repeating the life cycle. The possibility that soredia from different specimens come together accidentally, all contributing toward the final complete thallus, is suggested.

Production of soredia and fragmentation are probably the most important methods of reproductions in lichens, since a considerable number of foliose, as well as a few crustose and fruticose species, seldom produce apothecia and spores. Fragmentation was noticed as a thin layer of wind-blown lichen fragments on the snow-crust above timberline on Mt. Washington (120). Rejuvenescence is quite common among species of *Cladonia* as the basal portion of the podetia die, and branching continues above.

Sexual (*Ascomycetes*). The sexual cycle of these plants is not clear, though the presence of sexual organs in some lichens has been well established. The male and female reproductive organs are known, respectively, as the "spermatogonia" and the "carpogonia". Spermatia are generally borne in the spermatogonia, though Bachmann (11) did not find this to be so in *Collema pulposum*. The carpogonium has a terminal branch or trichogyne through which the spermatia must travel to reach the female cell. The spermatia, being non-motile, are transported by mechanical means, that is, dew, rain or insects, and become lodged on the trichogyne with which they fuse (plasmogamy). The male nucleus wanders into the trichogyne and then backwards toward the ascogonial cell.

Moreau (149) points out that it is the custom to distinguish between two main types of Ascomycetes. One, like *Pyronema,* has a coenocytic ascogonium which copulates with a multinucleate antheridium. *Peltigera* and *Solorina* possess ascogonia of this type. Other Ascomycetes possess an ascogonium in the form of a winding thread with uninucleate cells. In such forms, fecundation is attributed to spermatia by the intermediation of a trichogyne, the extremity of which is extended above the surface of the thallus. This has been described by earlier workers and by Bachmann (11), working on species of Collemaceae.

In a later paper Moreau (150) reported that in *Collema nigrescens* the trichogyne is not a constant organ, as Stahl stated in 1877. In later stages of development the ascogonial cells grow, acquiring two rather than several nuclei. This makes room for branching hyphae with binucleate cells. The latter branch off, forming asci at their extremities. Spermatia are said not to take part in this development, but rather ascogonia give rise to ascogenous hyphae without having undergone fertilization. In the light of recent investigations of *Neurospora* by Backus one should not unqualifiedly accept Moreau's findings. The whole matter of spermatial fertilization in a great number of lichens is highly probable, though few have observed the process in its entirety.

The reproductive cell, or ascus, is contained in one or two types of fruit bodies. In the Discomycete type the hymenium, or spore-producing layer, consists of asci intermixed with parallel packing hyphae, or paraphyses, and is fully exposed as a lining to a shallow, cup-shaped, fruit-body or apothecium. In the Pyrenomycete type, the asci are contained in a small flask-shaped structure, or perithecium, which opens outwardly by a minute opening or ostiole. The ripe ascus, usually containing eight spores, is a turgid, much elongated cell which forcibly discharges its ascospores into the air usually, as Werner (225) found in ascospores of crustose, fruticose and foliose lichens, from March to June. Hilitzer noted that in *Solorina saccata* spore discharge is dependent on the humidity of the thallus, especially of the lower cortical layer of apothecia which, when soaked, expands and exerts great pressure on the asci. Unlike most other Discomycetes, this lichen is dependent on heavy rains for its spore discharge. Production and liberation of its spores are continuous throughout the life of the apothecia.

Germination of Spores. On germinating, the ascospore is at first without the algae which it requires as gonidia, but when it comes into contact with its own particular alga, the fungus is stimulated to form a lichen thallus. The fungus is then able to grow independently of an organic substratum, as the green alga supplies the necessary organic food. The fungus absorbs water quickly, conserves it by reducing transpiration from the lichen surface, and thus furnishes its partner with moisture for a longer period than it would otherwise have at its disposal. In short, growth of the alga depends on the effort of the fungus which in turn is stimulated to greater activity by the metabolic action of the alga.

Determination of the exact relationship between the symbionts at this stage is of great interest. Cengia-Sambo (21) maintains that an oil, elaborated by the algal cells, is the first product of photosynthesis to be translocated to the hyphae of the fungal component. She further found (23) that in gonidia of Cyanophyceae the first product is glycogen; in the Chlorophyceae, it is an oil. Mameli-Calvino (137) could not affirm this, since in her research, starch was apparently the first substance produced, varying seasonally, disappearing in long periods of darkness, and present in and outside the gonidial cells. The latter conclusions agreed with those of Tobler (212) who further found that the fungal hyphae were able to excrete an enzyme capable of hydrolyzing this starch *in vitro*. The starch external to the algae first disappeared when the plants were placed in the dark. In the lichen, the alga does not outgrow the fungus under normal conditions. Since the equilibrium between the algal and fungal components cannot always be maintained in the laboratory, we have a partial explanation for failure to secure successful synthesis of lichens under artificial conditions.

Thallus Formation. Werner (226) has observed the thallus formation of *Xanthoria parietina* and other species. The filaments, developing from the germinating spores, intertwine on finding free-living algae, forcing themselves between the algae and the substratum. As the alga increases in volume and divides, certain hyphal filaments separate the daughter gonidia, while others increase the colony by finding new algae. A cortical layer is formed around the gonidia and this enlarges so that the young colony takes on the appearance of a small thick "leaf". Gonidia become localized among the hyphae under the upper cortex and at the same time a medullary layer and lower cortex are formed, eventually producing elongated rhizoids at the periphery of the thallus. During the last phase the development of the cortex is oriented, and the hyphae of the medulla, by added growth, displace young gonidia so that they come to be located in the new parts of the cortex where algae are lacking. Thus it would appear that in the development of the lichen thallus, the fungal element is active whereas the algal unit is relatively passive.

Systematics

Although lichen algae have been referred to free-living genera, the fungal component, for the most part, cannot be compared to genera of fungi not found in lichens. Modification of the latter, as previously indicated, has reached a point where there is often little resemblance between them and the ancestral forms. It would seem that both fungi and algae should be classified separately. If attempted, this would involve a new classification and nomenclature of the lichen fungi. In a sense, this is being done, since the apothecia and spore characters of the fungal element are used extensively in subdividing the lichens into subclasses, orders and species, while the algal component contributes to the arrangement of families and genera. Elenkin (51) proposed a system based on the essential types of fruiting bodies and habit of the lichen. On this basis he presents a chart showing the evolution of these plants and dividing them into normal families. Thomas proposed that the generic name of the lichen be provided with the ending "myces" and that the species name be placed in the genitive. Thus the lichen fungus of *Cladonia pyxidata* would be called *Cladonia-myces pyxidatae.* On the other hand, he would discard "gonidia" as a term for the lichen algae because of its vagueness.

Systematic lichenology is still in a hesitant stage. Authors with differing views constantly shift doubtful species from lichens to fungi and back. This is understandable when dealing with borderline species. For example, Mameli-Calvino (138) reports finding an epiphyllous Deuterolichen[10], *Chlorocyphella subtropica,* the fruiting body of which is a gymnocarpous receptacle inside of which conidia are produced. Though the perfect stage has not been reported, she unites it with the related species of *Chlorocyphella.* Martin regards lichens as fungi "eventually to be distributed among other fungi".

Magnusson (133) has criticized lichenologists for emphasizing one or two characters while equally important ones are ignored. Habits of growth and the presence of soredia or isidia are not, in general, sufficiently constant to be of great value in delimiting genera or species. $Ca(OCl)_2$, KOH, KI, HNO_3, paraphenylenediamine and other chemical reagents have been used to differentiate not only species, but varieties and a host of forms. Du Rietz (46) had earlier spoken against those who use chemical reactions as the basis of lichen systematics. Indication that the use of these reagents may lead to erroneous deductions was shown by Hesse (84) who reported that usnic acid was not a constant constituent of *Evernia prunastri,* since he had failed to find it in different samples of this lichen.

Gyelnik's use of unreliable morphological characters and chemicals in examining the Peltigeraceae has led to anything but clarity in that family. Scholander objected to Gyelnik's choice of variable chemical differences as diagnostic characters where morphological differences were lacking in founding species, varieties and forms. He states that: "Our knowledge of the physiology and chemistry of the lichens is insufficient, as is also the dependence on the substratum of their chemical substances. Neither do lichenologists generally know much of the chemical substances upon which they react. The result is that chemical characters can never be more than a help to distinguish between specimens which are morphologically defects or untypical. . . . By an assiduous use of KOH, $CaCl_2O_2$, Iodine . . . we could certainly increase immensely the number of species in genera".

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[10] *i.e.,* Lichen Imperfectus.

Asahina's (6) studies on the color reaction of the lichen thallus to chemical reagents, based on innumerable analyses of the chemical nature of lichen acids and other substances, led him to conclude that chemical components are as important as morphological characters for purposes of lichen classification; such chemical reactions may be of value in specimens which are not well developed morphologically. In the Cladoniaceae, this practice has resulted in a multiplication of forms, consisting of slight variations, and resulting in no useful purpose. Yet Evans calls for a more exact usage of the terms "form" and "variety" in this already large family of lichens.

Literature

There is greater need for monographic studies, not only of families and genera, but of species. Zahlbruckner's (232) general treatment of the lichens of the world brings together a vast fund of information and synonymy which is available to the monographist. Such studies are necessary not only because of the present day uncertainty in regard to specific limits, but from a phytogeographical point of view which may well contribute facts on the distribution of phanerogams. An example is furnished by Darbishire (34), who in monographing the commercially important Roccellaceae revealed that the Old World species differed.

The more recent monographs on lichens include those of *Acarospora* (132), Cladoniaceae and Umbilicariaceae (68) and Physciaceae (130), as well as papers on the Usneaceae (153), on *Lecidea* of New York State (122) and on *Parmelia* (14).

Greater cooperation between lichenologists of the Old and New Worlds is fundamental; this would include an exchange not only of type specimens, reprints and ideas but of teachers and students for a better understanding of the many problems of lichenology. One such perplexing question is the conception of lichen species. For example, with reference to *Parmelia tubulosa* (Hag.) Bitter, which has been found by the writer on Mt. Desert Island and occurs commonly in other areas, Degelius (42) states: "This species in the United States is too frequently overlooked. It is not mentioned at all in Fink's 'Flora'". Dr. Berry evidently did not find it in the exsiccatae and collections studied, because it was probably not differentiated from closely allied species. Degelius (42) reports *Evernia mesomorpha* Nyl. as: "On the coast one of the most common lichens on coniferous trees . . .". With one exception, no reference has been made to the occurrence of these lichen species in recent contributions to American lichenology.

It is difficult for the individual student of lichens to secure a manual that will not only present him with a survey of the lichen flora of his country but will also have critical descriptions and comparisons with similar species in other countries. Smith's "British Lichens" and Fink's "The Lichen Flora of the United States" are two works available in English to the more advanced student. Detailed microscopic structure and critical comparative studies must be obtained from monographs or from papers in the various publications. More recently, Nearing has published the "Lichen Book" which is not intended for the specialist but because of its many figures and field characteristics, is a much needed guide to the American beginner in the study of lichenology.

Distribution

The problem of the geographical distribution of lichens is complicated by many factors and by lack of sufficient studies. The peculiar association of two different plants forming a whole, presents an element of uncertainty, for in reproduction only the fungal component is represented, and this must meet the particular alga with which it is associated. Since lichens are also able to perpetuate themselves asexually, and since they are adaptable and resistant to unfavorable conditions, they are found widely distributed.

Paleontological. Paleontology has contributed little to the problem of present day distribution. Fossil remains of lichens have not been found earlier than the Tertiary. The lichen thallus decomposes rapidly and when found, even in humus, is apt to be fragmentary. Yet Sernander (191) has noted remains in postglacial peat beds and on partly fossilized tree trunks. Deecke has found *Cladonia rangiferina* in loess of the upper Rhine region.

In general, we can suppose that where vascular floras have been known to exist, lichen floras may well have existed, in spite of the scarcity of fossil records. Correspondingly, lichens as well as phanerogams were probably pushed southward by the advancing ice of the last glacial period. Lynge (129), however, suggests that some may have existed in alpine refuges or along ice-free coast lines, noting that today some hardy arctic plants are found on nunataks.

Regional.

a. Arctic. In this respect, the investigations of the Scandinavians are of interest, for they have done considerable work on the Arctic element in lichen floras of temperate regions. Lynge (124) states that though microlichens are well represented in the Arctic, it is the macrolichens that appear to have the greatest distribution. In revising the genus *Rhizocarpon,* he (126) found that though there appeared to be a characteristic regional difference in the distribution of the group, only six were circumpolar. In a later paper, Lynge (128) states that many lichen genera grow at an unexpected elevation; that many of the commonest species have a wide and circumpolar distribution. In his studies of Novaya Zemlya (125), he records collecting 413 species of lichens as compared to 155 species of vascular plants. In a collection made in East Greenland, Lynge and Scholander found a lichen flora of only 102 species. He (129) concluded that the Arctic has a true lichen flora and not an extenuated southern flora, that this appears to be very uniform, and has been possible only because many of the species are very old in their present or adjacent areas, and because of their great conservatism.

b. Antarctic. Surveys of the Antarctic element in lichen floras of the South Temperate regions have revealed parallel examples. Du Rietz (47) found in the high mountains of New Zealand a great number of species identical with or at least nearly related to Arctic lichens. Some were not found at any other place in the southern hemisphere; others were noted in the Magellan region. This Magellanic distributional area is traced to the Boreal regions by a series of localities along the Andes. He records known lichens which have a distributional area connecting New Zealand with the Magellanic area through the Antarctic. This may account for the presence of arctic elements in the flora of both South America and New Zealand. It also suggests the possibility of a migration route from Arctic to Antarctic by way of high mountain chains connecting these two regions during a period of uniform climate.

The New World and Russia alone have such north-south avenues which may provide evidence for this theory of distribution, but from a lichenologist's point of view, neither has been investigated.

Darbishire (35), comparing the Arctic and Antarctic lichen flora, pointed out their remarkable similarity in having in the species of both areas, a high percentage of fruticose lichens, which he considered evidence of their greater antiquity. Most of these are characterized by a very dark thallus. Hué, however, working on French Antarctic collections, found and described so many new plants that he concluded the lichen flora of Antarctica to be unrelated to that of any other region. Dodge, studying the lichens of the Byrd Expedition, reported that out of a possible 33 bipolar species, only one had been collected by the expedition, although 89 species in all were gathered of which 84 were described as new by the author. Lynge (131) has written a most interesting and exhaustive account of *Neuropogon sulphureus* (König) Elenk. [*Usnea sulphurea* Th. Fr.]. The genus is bipolar, being represented in the Arctic by one species and in the Antarctic by related species with intermediate localities in the high mountains of Ecuador and Peru.

Notwithstanding the fact that bipolar lichens have been found and that means for their distribution between these regions are available, it is quite possible that many lichen species have little capacity to migrate. This is brought out in collections made by a Finnish expedition in South America (171). Unless the continental drift theory of Wegener is accepted, it may be assumed that, for example, New Zealand and North American forms reached their present location along the narrow Cordilleran Chain since the rise of the Andes and the Rocky Mountains, which, considering the probable slight migration capacity of these plants, would appear unlikely. Yet the absence of bipolar species from tropical and occasionally from warm temperate zones is one of their best characteristics. For a more comprehensive study of the problem, see (48).

The idea that alpine vegetation might show an affinity with Arctic vegetation has occurred to many workers. In the United

States, Kiener lists 13 species of Peltigeraceae found in or close to the alpine zone on Long's Peak, Colorado, which he presented as such evidence. Llano advanced a similar view on the basis of a study of the lichen flora of Mt. Washington, New Hampshire. The genus *Cladonia* is well represented in the northern regions. Vainio enumerates 45 species of this genus in Finland. Savicz records 37 species in Kamtschatka, all part of the "circumpolar" group.

In *Acarospora,* Magnusson (132) found the genus to be cosmopolitan. These are lichens characteristically upland, some restricted to granitic or silicious substrata, but many found on calcareous rocks or earth. Their distribution was stated as follows: Europe, 100; Asia, 33; Africa, 39; North America, 71; South America, 19; Australia, 8; Antarctic, 3; Arctic Circumpolar, 1. Six were found common to North and South America and one common to the United States and the Antarctic.

c. Subarctic and North Temperate. Similarly, Suza records an arctic lichen, *Nephroma expallidum,* in Czechoslovakia and compares it with other arctic lichens in central Europe but not the Alps. Motyka (152), working at a lower level in the same region, revealed an astonishing similarity between the lichens of this area and those of the hilly region of the British Isles; the group *Cetraria* alone furnished 13 species common to both regions. On the other hand, Knowles has recorded tropical and subtropical species of lichens in southwest Ireland.

d. Tropical. The many climatic changes of the tropics have produced a greater abundance of species and individuals than is found in temperate and arctic regions. Northern species attain a larger size and certain families are especially abundant. The crustaceous groups, Thelotremaceae and Graphidaceae, are plentiful. Vainio, in a survey of Philippine lichens, has listed 635 species of which 178, or 28%, belonged to the tribe Graphideae, so that he concludes that this group reaches its maximum development in the tropics and will not be easily displaced from this position by additional discoveries.

Cengia-Sambo (24) has listed 60 species from Kenya and Tanganyika, noting that the lichen floras of these two areas show considerable similarity and relationship with the lichen flora of the Malayan Archipelago and the Philippine Islands. The genera

Parmelia and *Usnea* were best represented. Of a collection made in East Africa (25), 44 were characteristic of tropical or subtropical climates, 29 of temperate, and 14 were cosmopolitan in distribution.

It has already been noted that the Hymenolichens, including the genera *Cora, Corella* and *Dictyonema,* are exclusively lichens of eastern and western tropical regions.

It should be remembered that *Trentepohlia* is an abundant alga of the tropics which forms the gonidia of many of these lichen genera. However, Zahlbruckner (231), in collating all records of the lichen flora of Juan Fernandez Island, noted some interesting dissimilarities. Of the total number recorded, 25% had various blue-green algal symbionts, while 15% had *Trentepohlia* for a symbiont. In a study of the lichens of Samoa, he found a further falling off in lichens combined with *Trentepohlia,* while the blue-greens remained in the majority, that type of lichen being peculiarly a denizen of volcanic islands. This is of further interest because of the fact that blue-green algae appeared to be one of the first forms of plant life on Krakatoa and in the Katmai peninsula after volcanic action had decreased (79). Zahlbruckner (231) concluded that the lichen flora of Juan Fernandez Island was partly Chilean and partly sub-antarctic American in origin.

Relict Areas. From investigations of the distribution of lichen flora, studies of relict areas have developed. Torrey noted 15 species of lichens occurring southward in isolated areas at high altitudes in the United States. A more interesting case was that of *Parmelia tiliacea,* a coast lichen, which was found inland on relict marine areas which were once ornithocoprous sea cliffs and rocks of the Littorina period. This has been described by Sernander-Du Rietz in Sweden. Schindler finds *Teloschistes chrysophthalmus* and *Anaptychia leucomela* of the upper Rhine Valley to be relicts of forms which had a much wider oceanic distribution in Europe at one time. Degelius (41), in a most authoritative and comprehensive treatise of 22 oceanic lichen species of Europe, describes them as having a wide distribution, especially in the lower latitudes. Though in Scandinavia the oceanic lichens live mostly under optimal conditions and therefore cannot be considered as relicts, he concludes that their distribution in the rest of Europe would indicate them to be relicts of a Tertiary population which was broken up and scattered during the last glacial epoch. The remaining species must have survived the epoch on some warm coast of southwest Europe. The history of this oceanic group is extremely complicated and their restriction cannot be due entirely to lack of effective means of distribution. Humidity, especially rainfall and fog, were locally important. Temperature was found to be less effective though mild winters at high altitudes play a significant part. Biotic factors were very important so that in favorable areas real oceanic communities were formed.

Since in East Africa Quaternary glaciation did not occur, Höeg chose Victoria Falls for a study of relict lichen epiphytes. He found neither a relict flora nor unusual types, even in the dense forest. The lichen vegetation proved to be poor in individuals and not rich in species. Lynge presents the many aspects of relict species in the "Dactylina Problem".

Origin and Evolution

The evolution of lichens is in many respects problematical. Church (28) saw little difference in structure between the Peltigeraceae and some fossil seaweeds, except that the lichen had lost assimilating cells which have been replaced, in function, by intrusive green or blue-green cells of algae. Miss Smith (196) aptly refers to these algae as "skinned seaweeds". In a later paper, Church (29) proceeds to trace the development of lichen fungus from seaweeds. After migrating from the sea, lack of nitrogen kept the plant impoverished, while the water problem tended to keep them small and restricted to short seasonal periods of growth. He concludes that lichens and fungi are evidently polyphyletic in origin.

Darbishire (37) objected to this theory, preferring to think of lichens as land plants entirely and descendants of simple fungi after their adaptation to parasitic and saprophytic land living. He bases these assumptions on the presence of air spaces or pores (188) in the thalli of lichens, which to him represents a long line of land ancestors, no matter how simple. In an earlier paper, Darbishire (36) stated that the evolutionary tendency of lichens seems to be along the line of increasing to the utmost the capacity for carbon assimilation. Galløe (73), in his studies of Danish lichens, concludes that they are derived from Oömycetes and that changes have taken place as a result of psychic and immaterial processes: "I am most inclined to the view that the faculty present in the organism . . . is of a psychic immaterial nature". Fink (58) early held that part of the lichens were evolved from Discomycetes and part from Pyrenomycetes with such genera as *Peziza, Patellaria, Hysterographium, Phacidium, Chaetomium* and *Sordaria* among those which may be closely related to the ancestors of the lichens. In this respect he made a point of discontinuing to publish in the "Bryologist" in favor of the periodical "Mycologia". In a review of Smith's "Monograph of the British Lichens", Fink (61) commended the work highly but criticized the author for adhering to the concept of the duality of lichens. Again in a later review (62) of Smith's (198) "Lichens", he believed it unfortunate that the author did not follow the modern and logical interpretation that lichens are fungi using algae as hosts.

Physiology and Ecology

Metabolism. Experimental research on the physiology of these plants has revealed some interesting facts. Becquerel gradually dried in air and then in a desiccator, thalli of numerous lichens. These were exposed to a temperature of 268° C. for one to seven hours. On being returned to room temperature the lichens resumed normal metabolism. He further found that at low temperatures these plants are in a state of quiescence. He concludes that in arctic regions they resist cold, not because of snow protection but because of gradual dehydration by cold winds. Smyth, working with *Peltigera*, found that air-dried lichens retain 5% of water content and when desiccated over $CaCl_2$, 3 to 4%. The rate of respiration increases with temperature, as well as with a rise in water content, up to saturation point. The rate of assimilation increases with light intensity up to about 22,000 lux with the temperature between 10° C. and 35° C. Similarly, Ellée, experimenting with the same genus, found that assimilation of carbon dioxide by lichens was dependent upon their water content, the maximum being obtained at saturation. The younger part of the thallus showed better assimilation powers than the older parts. Measurements of light penetrating the lichen cortex indicated that about one quarter of the available light was able to pass through the gonidia layer when wet and only about one tenth when the thallus was dry. Stålfelt, in a paper concerning gas excretion of lichens, found that lichens depended upon light and temperature for gas exchange, as with most plants; the total amount of light required for photosynthesis was high for the species studied. The optimum range of apparent photosynthesis was not sensitive to changes between 0° C. and 15° C. The ratio of apparent photosynthesis to dark respiration increases with decreasing temperatures. Thus, during winter, photosynthesis is intensive and without waste; during summer, photosynthesis is extensive with appreciable waste.

Neubauer found that the water content of lichens varies from 2 to 10% of dry weight on dry days to over 300% on rainy days. The respiration of lichens is two to three times as much when wet as when dry. Cuthbert, by the use of sensitive electric apparatus, found that *Teloschistes flavicans* is metabolically active with a water content as low as 0.4%. He proved experimentally its great facility to absorb vapor from the atmosphere surrounding it, and this together with the ability to survive with an exceedingly low water content, gives an explanation of the drought-resisting power of this plant. Bachmann (8) noted that crustaceous lichens absorbed more water, weight for weight, than foliose forms. On the other hand, they lost more water to begin with. Endolithic lichens were most retentive of water. He concludes that the presence of a layer of dead hyphae and gonidia is of importance in absorbing, retaining and storing water.

Rate of Growth. Rate of growth in lichens is somewhat debated. Cooper, in a 17-year study of phanerogam succession, incidentally noted no apparent change in lichen colonies in the Lake Superior region. Fink (60) carefully established quadrats and measured lichen growth of different species. He found it undesirable to draw any conclusions. Still, he noted that certain crustose lichens became established and produced thalli and apothecia in denuded areas in two to eight years. Foliose lichens increase in diameter from 0.3 to 3.5 cm. per year. *Cladonia* regenerates squamules in one or two years and podetia in three or four years, and these plants become established in succession in four to eight years. It appeared to him that on the studies of primary succession, full development of apothecia to the point where they assume their mature forms requires from four to eight years when the plants are growing in their natural habitats. A much shorter time has been reported for development in cultures. The writer has studied specimens of *Teloschistes ramulosus* on *Thuja* twigs from Idaho, which had produced apothecia and spores in one year, as was ascertained from the fact that the thalli occurred on the previous year's leaves.

Effect of Substratum. Bachmann (10) found that some crustaceous lichens growing on bone did not draw any nutrition from the osseous tissue but that they benefited from the porosity of the substratum because of its great capacity of absorbing and retaining water, to the advantage of the lichen.

Mellor noted pitting by lichens on old church windows resulting from the mechanical action of the thallus. Fry (70) attempted to explain the mechanical force exhibited by lichens in the disintegration of rock. She found that films of gelatin, spread upon glass and dried, contract and tear away flakes of glass. Similar results were obtained when films of gelatin were dried upon smooth unweathered pieces of shale. The lichen thallus when wet is mucilaginous or gelatinous, and on contracting when dry, strips off thin sheets of stone in the same manner as drying gelatin. Rhizinae, rootlets and haptera of lichens appear to be mainly for attachment and of little significance as organs of absorption (198). Disintegration of rocks by lichens is therefore only partially due to the solvent effect of acids or excreted carbon dioxide; it results mainly from a mechanical action resulting from drying. Similarly, in studying the mechanical action of corticolous lichens (71), the bark is pulled up and broken by the shrinking of the thallus.

Ecological Factors. The number of important papers that have appeared on lichen ecology and habitat is indicative of the fact that lichens may be found everywhere except near the larger cities.

Frey and Ocksner account for the comparative richness of the lichen flora of central France by regarding it as the result of the greater uniformity of rock formations and forest conditions and partly as a result of the influence of oceanic climatic conditions. A rich growth of earth lichens was associated with a poor growth of epiphytes, and conversely. Lichen associations are best developed in luxuriant mats consisting of the fewest number of species. Exposure to sun, wind and available moisture are stressed as biotic factors. Davy de Virville pointed out that the lichen flora of the coast of northern Portugal is similar to that of the Atlantic coast of France, but that lichens become less abundant toward the south because of a difference in the geologic constituents of the rocks and more intense solar radiation.

Nutritional Factors. Sernander (190) early recognized the influence of abundant nitrogen on the growth of certain lichens. He classified as "ornithocoprophilous" those forms which occur on rocks frequented by sea birds, and as "coniophilous" the forms found along roads where dung-laden dust was abundant. Degelius (40) found that the lichen flora of islands in the Kattegat was greatly influenced by the abundant bird life there. Lynge (128) emphasized the great importance of bird droppings on the arctic coast in the development of rich lichen flora. He conversely noted that the sparseness of lichens in northeast Greenland was due, not only to the dry climate during the time of vegetation, but to the absence of sea birds. Some lichens show a definite preference for calcium-bearing rocks and are recognized as being calciphilous. Among these, however, it has been found that some will grow on rocks having a slight trace of calcium but with (36%) magnesium oxide (179).

The work of some Europeans appears to affirm the presence of nitrogen-fixing bacteria in lichens. One such case of polysymbiosis has been noted in cephalodia of lichens (23). Iskina, using Winogradski's silica gel technique, reports finding nitrogen-fixing bacteria in *Cladonia rangiferina,* suggesting that these bacteria may have produced the "intragonidial wart-like swellings" previously described in these plants (33). The paper of Iskina throws some light on the nitrogen availability of those lichens inhabiting localities apparently lacking in nitrogenous substances. Zakharova reports that addition of "bios", extracted from yeast, stimulates the growth of these plants two to three times. He suggests that the presence of nitrogen-fixing bacteria within the thallus produces a similar "bios" which, with the carbohydrates evolved by the algal component, is utilized by the lichen fungus and influences growth and reproduction, controlling the stability of the plant.

Trümpener maintains that pH is an ecological factor of prime importance in the distribution of lichens, but he acknowledges that the nitrogen content of the substratum and air is important in certain species. He found nitrophilous lichens most abundant at the base of trees and noted that the pH value tended to become lower, the higher they are taken on the tree.

Associations. Raup, in an investigation of the lichen flora of *Picea canadensis* (Mill.) B.S.P., found a distinct association. Crustose lichens occurred on the younger part of trees and branches; foliose, on older trunks, while a transitional region lay between the two. Important factors in the development of this association were age of the tree, roughness of bark, and exposure. Rassadina, from the structure of lichens distributed on tree trunks, concluded that their development is weaker on conifers than on deciduous trees. On deciduous trees, except birch and oak, he noted a difference in the grouping, depending on whether the bark was smooth or rough. He found that lichens usually occurred on the upper part of the trunk, regardless of the cardinal points. Haugh differs with Galløe (73) concerning development of lichens in beech forests. The latter stated that the poor lichen flora of beeches is due to deficiency of light in late winters and spring, resulting partly from the presence of dead leaves on the trees. The former concludes that optimum sites for growth of beech are poor in lichen growth because the trees grow rapidly, thereby shedding outer layers of bark often and at the same time forming dense stands which allow insufficient access of light. In ordinary forests, the slower growing trees carry more lichens.

Plitt (168) shows that the lichens of Mt. Desert Island and Blue Mountain indicate a definite competition resulting in a noticeable succession of crustose and foliose forms followed by fruticose species. The exact species to succeed is determined by the closeness of the attachment to the substratum, the less closely attached ones being able to overgrow the others. Moreau (151) observed the same thing in France, noting the more abundant crustose species on wind-exposed surfaces.

Lichens are plants characteristic of exposed, windswept and unfavorable habitats, prominent in alpine and arctic areas; they are equally at home on cold, foggy coasts and on trees of the tropics; along lake shores, in brooks, between tide marks, while one species has been secured from the Baikal Sea (92). They are considered pioneers in the scheme of plant succession, though they may actually form a permanent stage and not just a step in an ecological sense. Their reputation as rock destroyers and formers of soil has been considerably exaggerated.

Under such conditions of distribution and habitat, lichens show many converging forms influenced, no doubt, by external factors as well as by individual variation. These factors and variations are so insufficiently understood at present that detailed splitting into habitat groups can do little but obscure the general geographical trends. For present purposes, the older usage of the terms "crustaceous", "foliose" and "fruticose", with formations named after the dominant form, would do much to simplify the problem.

CHEMISTRY OF LICHENS

In the past 50 years about 200 compounds have been extracted from various species of lichens, mostly by German, Japanese and Italian workers. These compounds have not been found in any other group of plants and are thought to be due to the peculiar morphological association in these plants. The chemical constitution of about 12 of these compounds is known, although the empirical formula of most of them have been published. With few exceptions, all contain oxygen and are acid or neutral.

Our knowledge of the elementary chemical composition of lichen products and of the constitution of the chemical substances in them is comparatively new; as a result of the later investigations of Zopf and Hesse, we have a better understanding of them. As early as 1808, Berzelius (15) discovered that many species of lichens on extraction with hot water yielded a gelatinizing substance which he named "Flechtenstärke" (lichenin); later investigators have shown this to be not a single substance but a number of related carbohydrates. The names of some of these substances are indicative of the species from which they have been extracted, as "evernin" from *Evernia prunastri* and "usnin" from *Usnea barbata*. Of late years, Karrer and his collaborators have characterized lichenin as the "reserve cellulose" of lichens and have found it in the seeds of various higher plants. Reference will be made later to the action of ferments, especially lichenase, on lichenin in lichens used as food by snails. Lichen starch (lichenin) is said by Haas and Hill to be chemically, but not physically, identical with amylose of starch grain. It is also said to be closely related to cellulose, having similar optical and chemical properties as cotton.

The constitution and properties of some of these substances (evernic, usnic, obtusatic, and olivetoric acid, also atranorine) have been found to be related. Some, as rangiformic acid, appear to resemble fatty acids; others have been named "depsides" by Fischer who found their chain compounds analogous to peptides produced by linking together similar hydroxyl groups. Tanning substances are an example of this category of compounds, thus suggesting Fischer's use of the term "depside" from the Greek "to tan". Naturally occurring depsides are found chiefly in lichens, *i.e.*, lichen acids.

Employment of lichens as raw materials in pastries, confectionery, food, and in the production of alcohol depends largely on the properties of lichenin. The presence of a certain number of phenols, acid-phenols, and acid-phenol-ethers together with other substances in extracts of *Evernia prunastri* and other lichens, forms the basis of their use in perfumery and cosmetics. The tinctorial properties of lichens, long used in dye industries, are for the most part derivatives of orcinols in species of *Roccella*. In 1934, Asahina reviewed the progress of the chemistry of lichen acids from the time of Zopf and Hesse. Since this represents the most recent summary, it has been referred to in this study.

The chemical substances never occur in great quantities. Their detection involves fine, complex techniques; production of lichen substances for commercial use is rather limited and specialized. At present, this information is most interesting from an academic point of view, yet basic to a more complete understanding of the economic possibilities of the class Lichenes.

ECONOMIC USES OF LICHENS

Food for Invertebrates

Smith (198) has secured from the literature a large number of records of invertebrates known to feed partly or wholly on lichens. This includes not only mites, caterpillars, earwigs and black termites, but also snails and slugs. These animals appear to feed on all but the most gelatinous ones.

More recently Schmid has observed the snails *Chondrina avenacea* Brug. and *Pyramidula rupestris* Drop. feeding on the endolithic lichens, *Verrucaria* and *Protoblastenia*. They obtained the lichen by corrosion and ingestion of the rock surface, grazing mainly on the thallus and apothecia. Excrement from these snails contained fragments of calcium carbonate and green algae cells, while the hyphae and dead algal cells were apparently digested. All this is of interest, since there is a widely current assumption that lichens are remarkably well protected against attacks from animals by reason of acid substances produced by them. Not a few "new" species of lichens have been the result of insect and snail ravages, further modified by regeneration on the part of the plant (181).

Experiments have shown that snails will feed on potatoes covered with cetraric acid and on acids, rhizocarpic and pinastrinic, poisonous to other animals, but will not feed on vulpinic, recognized as poisonous to vertebrates (198). Crystals of the acid pass through the alimentary canal unchanged and are deposited in slime-covered masses in the feces. Bitter-tasting lichens, treated by a (198) soda method to extract the acids, were acceptable to many animals in preference to moistened, fresh lichens. Dry hard lichens are rarely attacked, while gelatinous ones offer a mechanical protection that gives them almost complete immunity from small animals. Algae are preferred in all cases, but when not obtainable the gonidia, *i.e.*, algal layers in lichen thallus, are resorted to for food. Hué goes so far as to state that the abundance of lichens in arctic regions results from the comparative absence there of snails and insects.

Lichens Used as Fodder

Non-grassy Ranges. North of tree line and above timber line there exist large irregular areas consisting of many square miles that may be classified as non-grassy range feed, covered with carpet-lichens serving in the capacity of grasses. The most continuous such areas are in the sub-arctic but these also extend into the north temperate zone and cover parts of Greenland, Iceland, Scandinavia, Siberia, Alaska, the Northwest Territories of Canada, and Labrador, as well as the many islands of the Arctic Ocean. In the Antarctic, lichens, though present, are not strongly developed because of unfavorable factors. Similarly, scattered and isolated non-grassy range lands are also present above timber line and well down into the timber of mountain sides.

The most useful species (198) are the so-called Reindeer Lichens: *Cladonia rangiferina, Cladonia alpestris* and *Cladonia sylvatica.* Zopf (198) states that the last is refused by reindeer. Included with this group are *Cetraria islandica, Cetraria crispa* and *Cetraria Delisei*...Probably others, as *Stereocaulon* spp. and *Alectoria* spp., are accidentally eaten, since they are to be found together. The Cladoniaceae are the most important, since they grow in carpet-like masses to a height of six or more inches. They appear to be less dependent on the substratum and grow equally well on all suitable areas, especially after fire, competing with and preventing development of certain seedlings. They may be covered for long periods by snow. Those animals that feed on them are accustomed to dig through this cover to obtain them. They support the reindeer which, in turn, make possible the existence of Lapplanders in northern Scandinavia. They are grazed by herds of caribou, wood buffalo, musk-ox and other animals in Arctic and Boreal America and provide an existence not only to the Eskimo and northern Indians, but to the white fur-hunters and traders. With the introduction of reindeer for the more stable support of Eskimos in the North, the value of these non-grassy ranges takes on new significance. A report of 1929 (200) by the United States Department of Agriculture in Alaska, where reindeer had been imported some years ago, states that there already is danger of over-grazing in this area. This is a problem that the Scandinavians have met with some success.

Use of lichens as fodder has always received attention in times of forage scarcity, while in some parts of the world they are regularly used for this purpose. Investigations concerning their food value and growing habits as well as the feeding habits of reindeer have been made at various times (121, 96). Lynge (123) presents the following interesting accounts.

In 1916, the large lichen fields of Finmarken maintained 100,000 head of reindeer which resulted in a serious over-grazing problem. The smaller fields in the Norwegian Provinces supported 50,000 of these animals. In order to remedy this condition, regulations prohibiting reindeer pasturing about Ravnastuen, Finmarken, had to be enforced for some time until good growth was reestablished. Under conditions of unrestricted grazing, lichen vegetation may be seriously altered, while mere trampling of large herds in small areas will also destroy these plants. In fields of *Cladonia alpestris, Stereocaulon paschale* comes in, producing full grown thalli in five to six years, after which *Cladonia alpestris* again becomes dominant.

In Lynge's account there is a list of Lapponian lichen terms contributed by Kristian Nissen, indicative of some of the peculiarities connected with reindeer husbandry. The Lapplanders, because of their association with these animals, have learned not to confuse mosses with lichens, since reindeer do not feed on the former though they will apparently take all accessible lichens. Lichen feed is classified under three headings. "Jaegel" refers to field lichens and includes the Cladoneae, Cetrareae and Stereocauloneae on which they fatten; "Gadna" or the Parmeleae and Gyrophoreae which occur on stones and trees and are eaten if no other food is available; while "Lappo" are the beard forms as Allectoreae and Usneae for which the animals have great fondness, and though these will support life, the reindeer do not fatten on them. The herders also recognize the pasture cycle after fire with its successive lichen formations. In all cases the reindeer prefer younger parts of the plants.

The relative abundance of these economic lichens would be best stated as "generally common", for solid areas of any one species is the exception rather than the rule. *Cetraria islandica* in average areas yields approximately 700 kilograms of air-dried "moss" per square kilometer. *Cladonia alpestris* gives higher yields, the richest fields being between Nordre Osterdalen and Finmarken with miles of lichen fields extending from the Swedish frontier to the Glommen valley. Selected areas here have produced 1400–1500 kilos per 1000 square meters.

Harvesting is performed by hand or implements. Among the Lapplanders, the work is done by women. Rakes or broad forks are used by men to clear the lichen vegetation away in broad strips, resulting in higher yields. The Lapponian method is more conserving and based on cheaper labor, since only a quarter of the quantity growing is gathered, leaving enough for regeneration. The Norwegian method takes up two-thirds of the amount available; this varies considerably with the person and the field. Dry "moss" is brittle and therefore most economically harvested when having a water content of 40–70%. As the plant is gathered, it is piled in small heaps (40–50 centimeters high) with a branch of birch in the center for a handle. The many small heaps are then brought together to form larger bundles. These are then taken to the drying house in sledge loads of from 300–600 kilograms, the bundles being gathered in the winter. Transportation costs are high. "Moss" presses are little used because of their expense and weight.

In the drying house the crop is spread out on the floor of a warm ventilated room, so that it does not lose too much weight, and is stored with a water content of from 14–15%.

A farmer having 10 cows and some sheep and goats uses yearly 60 sledge loads of lichens for his stock. This implies a need of 4,800–18,000 square meters of well covered lichen fields a year. Since these plants require 30 years to regenerate a marketable stand, a farmer must have access to 150,000 to 560,000 square meters of land. This land must preferably be mountain land, since forest areas contain pine needles. However, few farmers give so much lichen fodder to their cattle, actual amounts depending on the amount of grass available. In "moss" districts, three to five sledge loads are collected per cow.

As an additional food for domestic animals, especially swine, lichens are of value, and Lynge recommends greater use of svinamose (swine-moss) for these animals. Jacobj found that young pigs throve better on a combination of reindeer moss and ordinary feed than with the latter alone. He also fed rabbits and hares with *Evernia prunastri,* after extracting the acids, with satisfactory results. Icelanders feed *Cetraria islandica* to their cattle and ponies. It has also been reported good for oxen (228), while the richness of the milk of the small cows of northern Scandinavia is attributed to this food (100).

Nutritional Studies. The nutritive value of these non-grassy range feeds apparently lies in their high lichenin (lichen starch) content and in the fact that they are less bitter than most lichens. Hesse (86) worked out a comparison of their sugar content with that of potatoes and found that for *Cetraria islandica* the proportion was 1 of potatoes to 3.35 of lichen; for *Cladonia rangiferina,* 1:2.5. The former has been found to yield 61% carbohydrates and other products of its hemicelluloses. The bitter principle, due to the presence of lichen acids in even the mildest of these plants, must however be removed in order to make the fodder palatable to animals. This is done by soaking them in water for 24 hours or by addition of potassium carbonate to the water for quicker action. Sometimes the lichen is mixed in hot water with straw and salt before being fed to cattle. It is this action which removes the fumarprotocetraric and other acids from the plants. Use of lichen fodder goes back into antiquity, as indicated by prehistoric remains found near Lake Constance in Switzerland (198).

Lichens Used as Food by Man

History of Human Use. From the earliest times the food of man has included lichens, sometimes as a delicacy, but more often as the last resort in the face of starvation. In either case, they are of little importance commercially, though Swartz quotes Hanstien, chief lecturer in the Agricultural School at Aas, Norway, who prophesied that lichens were destined to become the great popular food for the masses, because of their cheapness and nutritive value. But the bitter principle of these plants gives them an unpleasant flavor, is difficult to remove, and exerts an irritating effect upon the digestive tract of man, causing inflammation. More recent studies in the field of experimental physiology have revealed some nutritional facts about these foodstuffs.

Cetraria islandica probably rates first as human lichen food. It was occasionally gathered commercially in the Scandinavian countries and in Iceland and sold on the market as Iceland Moss. Preparation includes removal of foreign material and soaking; then it is dried and powdered. It is made ready for human consumption by boiling as a broth which on cooling sets as a jelly. Milk is added and in this form the lichen is the basis of various light and easily digested soups and other delicacies said to be of value for dyspeptics. It may be made into bread, porridge or gruel. Schneider says of this "moss": "Inhabitants of Iceland, Norway and Sweden mix this with various cereals and mashed potatoes from which an uncommonly healthful bread was prepared". Lynge (196) quotes a tradition "that there was no starvation at Modun in 1812 as long as there was bread-moss (brødmose) left in the forest". It was also mixed with ship's flour, making the bread less friable or less subject to weevil attack. In northern Finland, in times of famine, reindeer moss and rye were made into a bread having a taste like wheat bran but leaving a sense of heat on the tongue. "Tripe de Roche" or Rock Tripe was so named by the French Courreur de Bois of Boreal America who used it in periods of emergency. Franklin (215) recorded it in his diary as the main course of many a meal. This "Tripe" is usually one of the Umbilicareae which must be treated by boiling or soaking before being eaten. The American Indian's knowledge of wild food plants included use of *Alectoria jubata* (230).

The biblical manna of the Israelites may have been *Lecanora esculenta* (198) which is still eaten by desert tribes, being mixed with meal to one-third of its weight. This lichen grows in the mountainous regions and is blown by high winds down into the lowlands where the thalli form little hummocks in the valley. As late as 1891 (Pharm. Jour.) there was an abundant fall of this "manna" in Turkey. The ancient Egyptians made bread, using *Evernia prunastri* and *Evernia furfuracea* (101). There is still some importation of these lichens from Europe as fermentative agents; Forstal in the 19th century saw several consignments from Islands of Archipelago for Alexandria (169). In India (198) a *Parmelia* species is used as food, generally a curry powder, and medicinally; while in Japan *Umbilicaria esculenta* is considered a delicacy and sold as "iwa-take" or "rock mushroom". Collecting places are scarce and difficult of access so that the market price is very high (105). In France, lichens are used in the manufacture of chocolates and in the pastry business, largely because of the properties of lichenin (158).

Nutritional Studies. Scientific investigations as to the digestibility of lichens and the behavior of lichen substances in the body have been too few, but the evidence at hand does not agree entirely with the fact that these plants are used extensively as food stuffs. Analyses have shown that they contain a variety of carbohydrates of which polysaccharides are the most common, giving rise, on hydration, to several sugars, some cellulose, chitosan, glucosamine and inulin. Of these the only compounds directly available in intermediate metabolism are the simple monosaccharides, *i.e.*, six-carbon sugars. Polysaccharides apparently need to be split into "physiological" sugars before they become available in the body. Ullander and Tollens noted a difference in the occurrence of characteristic carbohydrates in various lichens examined, though they all contained some lichenin. Thinking that the substances in *Cetraria islandica* and *Cetraria nivalis* were similar, Poulsson made a bread from these two species to determine their use in diabetes mellitus. Though 46–49% of the carbohydrates of the former species was digested, the latter species caused such intestinal disturbances that the experiment had to be discontinued. Brown failed to induce glycogen formation in rabbits by feeding them lichenin obtained from Iceland Moss. Ordinarily neither emylytic enzymes nor hydrochloric acid (0.3–0.5%) have any noticeable effect on lichenin, while iso-lichenin is, at most, converted into a dextrin-like form without producing sugar; the action of bacteria yields acetic, proprionic, butyric and lactic acids (102).

More recently Wallerstein fed mice white bread, later replacing it with lichenin, and showed the latter to be 53–64% utilized. Similarly, Shimizer (193), in determining the influence of some polysaccharides on the protein balance of a dog, found that they were digestible and available foodstuffs in the alimentary canal; though how, he did not state. Later (194) he digested polysaccharides *in vitro*, using extracts of macerated intestine and pancreas in an 0.8% NaCl solution but found no monosaccharides. He took this as evidence that there are no enzymes in the digestive system of mammals capable of splitting inulin, lichenin or hemicelluloses. On determining the action of fecal material and fermentative bacteria on these substances, Shimizer and Tonihide (195) concluded that they are split into sugars by the bacteria in the digestive tract of mammals and can then be absorbed.

It has been assumed in the past that the presence of the enzyme lichenase in the stomach contents of the ox and pig probably enable these animals to convert lichenin into the more digestible sugars (103). The action of snail lichenase on lichenin *in vitro* has been found to produce cellobiose and lichosan, an anhydride of glucose similar to cellosan, a product of cellulose. Messerle states that the livers of snails contain much lichenase which converts cellulose to sugar. Jewell and Lewis had found this to be true of many invertebrates, suggesting that the ability to hydrolize lichenin may be characteristic of invertebrates only.

Swartz questions the value of algae and lichens as sources of energy in nutrition. Oshima suggests that they may be valuable for their inorganic salts, while Prausnitz calls them "faeces-forming foods" in that they stimulate intestinal activities. Most of Swartz's studies were on the algae, yet she was able to draw certain conclusions concerning those chemical substances common to both plants. These were:

a) Nutritive studies of lichens would indicate that as energy producers their value is not appreciable. Yet the fact remains that certain animals do feed upon them and thus sustain themselves in regions where energy and high body heat are prerequisites to life. (The order of resistance of hemicelluloses to bacterial action was noted by Saiki—as galactans, pentosans, levulans and mannans —and herbivores were found to do better on pentosans, not at all on mannans.) The assumption follows that our understanding of the value of lichens as fodder is still incomplete, though ruminants are apparently more effective users of hemicellulose than other animals.

b) Aerobic and anaerobic bacteria, not enzymes, are responsible for conversion of hemicelluloses into sugars. The amount available to the animal system is extremely diverse, depending on the animal and the lichen; in dogs the ratio of digestibility of pentosans varied from 25–75.

Vitamin Studies. Nutritionists have assayed lichens from the vitamin point of view. Blix and Rydin found that *Cladonia rangiferina* contains some ergosterol, more than most lichens, but the content is low in comparison to yeasts and molds. This is a substance similar in composition to cholesterol. This same species collected in Upsala in August and September showed only traces of Vitamin D.

Feeding experiments with rats failed to show Vitamin B or G in either of two samples of short and tall lichens obtained in Alaska (54). Short-growth lichens gave more Vitamin A and less Vitamin D than tall-growth types. Short-growth types appeared more palatable to rats. Unfortunately, in this experiment the names of the lichens used were not indicated, so that this evidence is only of the most general interest. Bourne and Allen, using acetic acid-silver nitrate reagent for Vitamin C, obtained a positive test in lichens.

Medicines and Poisons Derived from Lichens

The name "lichen" is a word of Greek origin meaning "leprous", used by Theophrastus in his "History of Plants" to signify a superficial growth on the bark of olive trees. The term originally applied to hepatics. Dioscorides applied it to true lichens on account of their resemblance to the cutaneous disease for which they were supposed to be specific.

History. Medicinally these plants can be traced back to a very remote age. *Evernia furfuracea* has been found in an Egyptian vase from the 18th Dynasty (1700–1600 B.C.). It is still sold in Egypt with *Cetraria islandica* as foreign drugs, being imported from Europe (198).

In the 15th century A.D. there was throughout Europe a constant attempt to follow the guidance of nature in the study and treatment of disease. It was believed that Providence had scattered here and there on plants "signatures" of resemblances more or less vague to parts of the human body, or to the diseases to which man is subject, thus indicating the appropriate specific. This era was the climax of the commercial importance of these plants, for never before or since have they played such a unique role in the world of economic plants.

The long filaments of *Usnea barbata* were used to strengthen the hair, though Hippocrates prescribed it for uterine ailments (114). The natives of the Malay Peninsula (66) still use this plant for colds and strengthening after confinement. *Lobaria pulmonaria* was the suitable remedy for lung troubles. Boerhaave (*Aphor. comment. de Van Swieten*) regarded it as an excitant, tonic and astringent and hence recommended it for hemorrhages and asthma. *Xanthoria parietina*, being a yellow lichen, was supposed to cure jaundice, while *Peltigera aphthosa*, the thallus of which is dotted with small wart-like tubercles, was recommended for children who suffered from "thrush". Other species of *Evernia, Peltigera, Parmelia, Cladonia, Roccella* and *Pertusaria* were used to control fevers, diarrhea, infections, skin diseases, epilepsy, convulsions and as purgatives. *Pertusaria communis* was especially interesting in that it was used to cure intermittent fever, having less action on women than men (114). *Peltigera canina*, as a cure for hydrophobia, was sold by a famous Dr. Mead as the celebrated "Pulvus antilyssus" (Dillenius 1741), while the so-called drug "Lichen quercinus virdes" consisted mostly of *Evernia prunastri, Evernia furfuracea* and *Parmelia physodes* (189). The doctrine reached the height of absurdity in the extravagant value set on a lichen found growing on human skulls, "Mucus cranii humani". This skull lichen, *Parmelia saxatilis* (*Physcia?*), as a cure for epilepsy, fetched its weight in gold.

Luyken, in his "Historia Lichenum in Genere", Göttingen 1809, gives a long list of medicinal "Lichenes, quorum usus obsoletus est". Yet as late as 1927 (219) there appeared in the Paris Medical Bulletin a discussion of the probability of true lichens being the cause of some skin diseases, with notes on therapeutic methods to be employed if laboratory findings should corroborate this. Plitt (167) recommended more emphasis on the study of lichenology to pharmacognosis, venturing the opinion that the medical virtues of bark drugs may be affected by the lichens growing on them.

Reference has already been made to lichenin and the many acid compounds present in lichens which give them a bitter and astringent taste. Some of them may have been of value as curative herbs, but all with the exception of one have been replaced by more effective modern drugs.

"Iceland moss" was given an important place in medicine by Linnaeus in 1737. It has been used in chronic affections as an emollient and tonic, and it would indeed have been a "Divine gift to man" had it lived up to all its prescriptions (31). Today it is used as a substitute for salve bases in the preparation of emulsions, the reduction of the bitter taste in certain drugs, as a laxative and as a culture medium for bacteria (166).

Physiology. The physiological action of the cetraric acid of "Iceland moss" has been studied by Kobert. It has no poisonous effect either when injected into the blood or when taken into the stomach of small animals. Small doses induce peristaltic movements in the intestines. Large doses may injure an animal, but if given as free cetraric acid, it passes through the stomach unchanged to become slowly and completely dissolved in the intestine. The mucous membrane of the intestine of animals that had been treated with an overdose, was found to be richer in blood, so that Kobert assumed that cetraric acid would be useful in assisting digestion. A more reasonable explanation might be that the lichen acid irritated the sensitive mucous membrane and produced inflammation. In this sense, cetraric acid would hardly serve as an aid to digestion. It has also been used as a nerve excitant.

It is interesting in this respect to note that Asahina and Fujikawa, in 1932, extracted (by means of acetone) from *Ramalina calicaris* *d*-usninic, evernic and obtusatic acids. The last named acid was the same as Makao obtained from the Manchurian drug Shi-hoa.

Lichens with few exceptions are non-poisonous, though their acid substances have proven extremely irritating when taken internally. The supposedly poisonous exceptions are the species *Evernia vulpina* and *Cetraria pinastri.* The former contains vulpinic acid in the cortical cells, the crystals of which are lemon yellow in the mass. The latter species and *Cetraria juniperina* (111) produce pinastrinic acid in the hyphae of the medulla and the crystals are orange or golden-yellow. These have been used in northern countries to poison wolves (198). Carcasses are stuffed with lichen and powdered glass, the wounds caused by the glass rendering the internal organs extremely sensitive to the action of the lichen acids. It would appear that the ground glass was sufficient to cause the death of the animals.

More recently a report of the Wyoming Agricultural Experimental Station (229) on a study of the presence of selenium in soil and various plants states that *Parmelia molliuscula* contains this poisonous salt in sufficient quantities to affect sheep and cattle. It produces a lack of coordination of the hind limbs; in severe cases the animals are unable to move either hind or fore legs. Other cases of lichens containing such elements include beryllium (57) in *Parmelia saxatilis* and *Xanthoria parietina,* chlorine (111) in *Evernia furfuracea.*

The physiology of lichen poisons has been studied (112, 156). Experiments on cold and warm blooded animals have proven fatal, the cat being the most sensitive of mammals and the hedgehog the least sensitive of those used in the tests.

Industrial Uses of Lichens

Brewing and Distilling. Use of lichens instead of hops for the brewing of beer is mentioned as occurring in Russia (114) and Siberia (215). Its use appeared to be confined to one or more monasteries in Europe which had a reputation of serving bitter but highly intoxicating beer to the traveler. Tuckerman further describes a by-product of *Lobaria pulmonaria,* "a yellow, nearly insipid mucilage which may be eaten with salt".

Alcohol production from lichens is an old art, more recently replaced by increased cultivation of potatoes and importation of sugar, better adapted to fermentation. Preparation of spirits from lichens was recommended in 1870 (201) as a means of saving grain otherwise diverted into alcohol production. This same writer claimed that 20 pounds of lichen would yield five liters of 50% alcohol. In 1893 (82) the manufacture of brandy from lichen alcohol had become a large industry in Sweden, though by 1884, as a result of local exhaustion of the plant, the industry languished. Arendt, in 1872, reported that this originally Swedish discovery was being applied in the Russian Provinces of Archangel, Pskow, Novgorod, *etc.*, and that various distillers exhibited samples of lichen spirits at the Russian Industrial Exhibition in Moscow, which were highly approved by the French and English visitors. The industry was a lucrative one in the northern provinces of Russia, yielding a net revenue of from 40–100%.

Lichens may vary in the amount of carbohydrates (lichenin) present. *Cetraria islandica* and *Cladonia rangiferina* have been found to yield up to 66% of polysaccharides which are readily hydrolyzed to glucose and then almost completely fermented to alcohol. Besides sugars capable of fermentation, lichen acids up to 11% of air-dried substance may be present. These acids (cetraric) as well as sodium chloride have been found to retard the process.

Experiments (54) with *Cladonia rangiferina* have shown a total yield of 54.5% sugar which on fermentation produced 176–282 cc of alcohol per kilogram of the plant. Maximum returns of alcohol were obtained by steaming the lichens one hour under three atmospheres pressure, adding 2.5% of 25% hydrochloric acid, resteaming for the same period of time and pressure, finally neutralizing the product. Subsequent growth of yeast was normal though fermentation may be accelerated by addition of H_3PO_4. An interesting modification of this procedure through addition of three parts by weight of sulfuric acid and one part by weight of nitric acid at room temperature, gave a pentanitrate similar to cellulose nitrate which, on gelatinizing with a solvent, produced a substance resembling horn (174).

Tanning and Dyeing. The tanning quality of lichens is due to an astringent property peculiar to some species (116). *Cetraria islandica* and *Lobaria pulmonaria* are most used and though not occurring in quantities sufficiently large to warrant industrial application, are locally employed on a small scale. "Many crustaceous lichens contain oxalic acid in greater or less abundance; according to M. Braconnet, this occurs in the bitter *Variolaria* (*Pertusaria* *sp.*) in such quantities that 100 parts yielded 18 of lime, combined with 29.4 of oxalic acid; and the lichen is now employed in France in the manufacture of the acid on a very extensive scale" (215).

The development of synthetic aniline dyes has largely replaced many common vegetable dyes. Of these, the lichen dyes were renowned for their high quality and color, some of which are still popular in rural districts of Great Britain and the Western Isles, Iceland, Scandinavia, France, Germany and the United States. The writings in 1787 of Amoreux, of Hoffmann who included in his "Commentatio de Vario Lichenum Usu" 77 samples with color names as used by the French "tienturies" of his day, and of Willemet reflect the part these plants played in the economics of the period. Westring in 1792 distinguished between lichen dyes which impart color to pure water (essential pigments) and those requiring certain treatment to yield color (preparable pigments). Lebail in 1853, Lindsay in 1854 and others had classified them according to the color produced, recognizing that color varied with treatment and that the hue of the plant *in situ* was no indication of the dye it might yield. The system followed by the writer is of a more general nature, based on records in the literature examined, some of which may be doubtful. Where possible, the nomenclature has been brought up-to-date (Appendix).

a) *History.* Of all the lichen dyes used by man, none has attained greater historical and commercial importance than those of the Roccellaceae, variously known to the English as Orchella Moss or Weed, Archil or Orchil paste or liquid, to the French as Orseille, and to the Germans as Persis. Theophrastus and Pliny both knew of the dye, while the Bible refers the origin to the "Isles of Elisha". During the Middle Ages the art of making dye from *Roccellae* fell into disuse and the dye disappeared from the markets of the world until the 17th and 18th centuries when it again took on the aspects of an industry, and the "Weed" became an article of international trade.

b) *Blue and Red Dyes.* About 1300, a Florentine merchant named Federigo (19), while in the Levant, noted that urine imparted a very fine color to some plants. On returning home, he experimented with successful results and founded the lucrative dye industry which established his family name, the Orcellarii, Ruccellarii or Rucellae, and gave to his native city a monoply that existed until the discovery of the Cape Verde Islands. This, or possibly the Spanish term for the plant, "Orcigilia", is thought to be the origin of the botanical name Roccellaceae. Though the early source of supply was the Levant and the Mediterranean countries through Florence, discoveries of new lands revealed the abundance of this lichen on rocks along warm sea coasts, and the trading centers became successively Montpellier and Holland. The Canaries, as well as the Cape Verde Islands, Cape of Good Hope, Angola, East Africa, Mozambique, Madagascar, Zanzibar, Ceylon, the East Indies and Australia; then in the New World, Valparaiso and Lima as well as the West Coast of North America, became the successive sources of the "weed". Importers have always been averse to disclosing the origin of their best supplies, but *R. tinctoria* of the Cape and South America was larger ("6–8 inches long, and as thick as goosequills") and so preferred by the trade (203). In 1750 the Cape Verde and Canary Islands exported 100 tons annually to England. In 1818 this cost from £40 to £200 a ton, depending on the quality (19), but by 1886 *R. tinctoria* gathered in Ceylon off palm stems had fallen to £50 a ton (143). The last figures available list the importation of tanning and dyestuffs into England for 1935 (88) as Annatto, 837,919 pounds; Brazilwood, 854,581 pounds; Cudbear (including orchil) Lichen dyestuffs, 411,265 pounds.

The chemical nature of *Roccella* dye was not understood in the early days of the industry and this was further complicated by trade secrets and tradition. In the old English method, the lichen was cut small or reduced to a powder by passing it through a sieve, and placed in iron drums provided with stirrers. It was then moistened slightly with stale urine, the mixture being stirred once a day with additions of soda for 5–6 days at a temperature of 35° to 45° C. Fermentation proceeded and was checked frequently until the coloring matter, a dove gray, ceased to increase. The product, Orchil Paste, was then placed in wooden casks and covered with a sufficient quantity of lime water or gypsum solution until needed by the dyer. To make Orchil liquor, the lichen was treated with water and urine and permitted to ferment as for Orchil Paste after which the fibrous matter was removed and the liquor collected and stored. Sal ammoniac, sal gem and saltpetre were sometimes used in the process (108).

Dillenius in 1741 said of its color "that it was reckoned more beautiful when first dyed, than the Tyrian Blue", while Bancroft (19) describes the infusion of Orchil as of a red crimson inclining to violet.

Modern methods were based on a more accurate knowledge of the chemistry of the lichen dye. According to Hill, the lichen is sprayed with ammonia in the air until the mass turns color, when the blue orchil liquor is extracted with water; if heated until the ammonia is driven off, red orchil results; afterwards the plants are dried and ground to a fine powder.

The French (19) employed a crustaceous species commonly called "perelle" to obtain a purple-blue dye. M. Cocq, in the 81st volume of the Annales de Chimie, describes its preparation as observed at Clermont, France. The lichen is macerated in wooden troughs, six feet long, three feet wide and two feet deep, fitted with tight covers. Two hundred pounds of perelle and two hundred and forty pounds of urine are mixed in this trough and stirred for three hours for two successive days and nights. The cover is removed only for stirring in order to retain as much as possible the volatile alkali (ammonia) of human urine. On the third day, ten pounds of sifted and slaked lime are added and well mixed together with a quarter-pound of arsenic and an equal weight of alum. The mass is then stirred repeatedly several times, once every quarter-hour; later, every half-hour until fermentation is established to prevent the formation of a crust on the surface. In forty-eight hours, fermentation is renewed by adding two pounds of sifted lime and stirring continually, once every hour until the fifth day. On the eighth day, it is stirred every six hours, extending the process a fortnight or three weeks. The coloring matter is kept moist in closed casks until used. It is said to improve the first year, suffers little change during the second year, and begins to deteriorate in quality on the third year of storage.

Dr. Bancroft (19) recommended the use of ammonia over urine and pronounced the addition of arsenic and alum as useless and dangerous while suggesting that hogsheads would facilitate agitation without the need of removing the cover of the container.

c) Brown and Yellow Dyes. Employment of brown and yellow dyes is an old custom in the northern countries of Europe. Fries remarked on the use of the class, Lichenes, in the Arts "that almost all that is known has been owing to the Northern—the Anglo-Saxon, Scandinavian and German Nations whom necessity constrained to value and improve all of Nature's gifts". In certain districts of Scotland as late as 1855 (115), almost every farm and cotterhouse had its tank or barrel of "graith" or putrid urine (the form of ammoniacal liquid employed) and its "lit-pig" wherein the mistress of the household macerated some familiar "crottle" (Scotch vernacular for lichens in general) to prepare dyes for their homespuns. The usual practice was to boil the lichen and woolen cloth together in water or the urine-treated lichen and cloth until the desired color was obtained. This took several hours or less on the addition of acetic acid, producing fast dyes without the benefit of a mordant or fixing agent. The dye substance had no deleterious effect on the wool. The color would be intensified by adding salt or saltpetre. This method was used not only in Scotland but also in Iceland, for those homespuns best known to the trade as "Harris Tweed" (78). Horwood (94) states that in the Shetlands the lichens are harvested in May or June or after rain in the autumn or winter, using a metal scraper for obtaining the rock species. These are washed, dried in the sun and sometimes powdered. To this powder is added quicklime and water, including ten pounds of lichen to a half pound of sal ammoniac and then permitting the mass to ferment in a covered vessel for a few days. These lichens were sometimes prepared for the London market and shipped in casks as "cudbear". The term is derived from a corrupt pronunciation of the name of Dr. Cuthbert Gordon, a chemist of Glasgow, who obtained a patent for his process in preparing the dye from *Ochrolechia tartarea* on a large scale. It is said that one person could collect 20–30 pounds daily, any one locality being visited every five years (19). The lichens were washed and dried, reducing their weight by half. The peasants of Westmoreland and Cumberland gathered this species for dyes at the rate of one pence per pound, while manufacturers of woolens and silks paid ten shillings a hundredweight for it with a profit to the middleman of eight pence. For storage purposes the lichen was mixed with aqua ammonia from human urine or pounded with stale chamberley, salt or kelp. The coarse paste was then rolled into small balls or cakes with lime or burnt shells. These were wrapped in dock leaves and hung up to dry in peat smoke, which accounts for the odor peculiar to "Harris Tweed". In this fashion, it would keep for a year or more and when needed it was redissolved in warm water.

The color of Cudbear and Orchil is so similar as to be commercially indistinguishable. They dye best in a neutral bath producing a bluish-red or dull magenta shade but were frequently applied with sulfuric acid in conjunction with other vegetable dyes and coal tar dyes, especially magenta (140). Addition of indigo and the dye of lungwort would give a permanent black dye; while *Roccella tinctoria* was used as the first dye for blue British broadcloth, having a purple tint against light. A variety of colors and shades could be obtained by the use of different species or varying the treatment with oil of vitriol, logwood or chemicals. Thus acids produced yellows, alkalies produced blues, lead acetate gave a crimson precipitate, calcium chloride a red precipitate, stannous chloride a red then yellow, while alum was more generally used by the country folk for reds. The color of cudbear was said to possess great beauty and lustre at first, but quickly faded and should never be employed unless for the purpose of giving body and lustre to blue dyes as indigo ("bottoming") or as a ground for madder reds (115). In deep shades, the color has an intensity and body which cannot be equalled by coal-tar substances, and though they are not fast to light or milling and scouring, they do resist soaping, becoming bluer (108). Silks, and occasionally linens, had the dye applied in a soap solution with or without acetic acid, and though still in use this method has been superseded in a large measure by synthetic dyes. Lichen dyes have not been applied to cotton cloth.

Cudbear and Orchil have both been used in Holland for the manufacture of litmus, known to the French as "tournesol". The dye is first prepared, after which gypsum or powdered chalk is added and the mixture cast into small, purplish-blue cubes once sold as "lacunus". This, when dissolved in water and soaked up in unsized paper, was retailed as litmus paper. The early product was rather unstable and tended to become colorless. This was thought to be due to micro-organisms, so that alcohol or chloroform was often added when the litmus was kept in liquid form. Tincture of Cudbear is still used in the drug trade, though since the Dutch source of supply is no longer available, it has been necessary for the U. S. Pharmacopoeia to substitute a coal-tar derivative, amaranth (187).

d) *Chemical Properties of Dye Lichens.* Dyeing properties of lichens are due to the presence of lichen acids, some of which afford the chromogens from which the coloring matter is derived. Keegan reports its development as an excretion in the outermost part of the lichen thallus and the upper side of the apothecia, or in general, those parts of the plant best exposed to the air at all times of the year and at all latitudes. This is of interest, since tradition would have the best colors produced from late fall harvests. The lichen acid occurs as an ester and a result of the symbiotic condition of these plants, the fungus component being the sole seat of the product, though Keegan says it cannot develop the acid by itself. He found that when the quantity of proteins decreases in the lichen the quantity of acid increases. As the alga borrows proteins from the fungus, a powerful process of oxidation is inevitable in the fungus. The alga would use the nitrogenous group of the protein molecule, the aromatic groups not needed would be left behind in the fungus. According to Keegan, then, the acids originate by a mechanism of deassimilation (oxidation) provoked by a penury of nitrogen in the vicinity of the algal cells; it would appear that formation of these acids is not as simple or direct as that of tannins or other chromogens of higher plants. The blue-purple litmus would be due to the addition of an inert nitrogen atom to the molecule which serves to check the otherwise natural tendency of orcinol on higher oxidation to assume a typical red or brown shade (106). In the Roccellaceae there are two different coloring substances, erythrin and lecanoric acid. When treated with ammonia, the acids are apparently split to orcin and carbonic acid, the orcin under the influence of oxygen, yielding orcein which is the coloring principle of orchil.

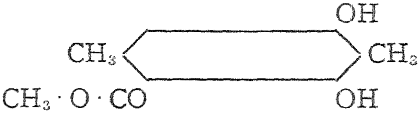
Cosmetics and Perfumes.

a) *History.* Since the 16th century, members of the families Cladoniaceae, Stictaceae, Parmeliaceae and Usneaceae have been used as raw materials in the perfume and cosmetic industries. At first this consisted of drying and grinding the plants to a powder and combining them crudely with other substances, but as the manufacturers became more expert in their trade, these materials were skillfully combined into toilet powders and scented sachets of high value. Amoreux (2) records the use of *Evernia prunastri*, though *Evernia furfuracea* and *Lobaria pulmonaria* possess similar aromatic substances and were also used. The lichens sought for these purposes were known under a variety of names, as "Lichen quercinus viridis", "Muscus arboreus, acaciae, and odorante", "Eichenmoos" and most commonly as "Mousse de Chêne" or Oakmoss and Scented-moss (77). *Ramalina calicaris* was used in place of starch to whiten hair in the dye of wigs and perukes. Cyprus Powder, a combination of *E. prunastri, Anaptychia ciliaris* and *Usnea* species, scented with ambergris and musk, oil of roses, jasmine, or orange blossoms, was celebrated in the 17th Century as a toilet powder that would whiten, scent and cleanse the hair (198). After a somewhat lengthy eclipse, these plants reappeared as raw stuffs for perfumery, owing to the creation of scents with a deep tone and to the demands for the very stable perfumes of modern extraction for which purpose they are almost universally used to this day.

The principal species used today in perfumes and cosmetics include *Evernia prunastri, E. furfuracea, E. mesomorpha, Ramalina fraxinea, R. farinacea, R. pollinaria* and others of the Ramalinae, though when this group is included in the mass it serves to depreciate the value of the former. *Lobaria pulmonaria* (Mousse de la base du Chêne) is used to some extent, though it is considered a more costly substance, perhaps due to the fact that in Europe it is confined to subalpine woods and is, therefore, scarcer (159). The true Oak-moss (*E. prunastri*) of Europe is collected in shaded, damp habitats occurring in all parts of France, especially in the central mountain ranges, in the Alps and in the neighborhood of Paris, the most valued coming from the Forest of Fontainebleu; also from the forests of Czecho-Slovakia, of Herzegovina and the Piedmont of Italy (93). Not only the source but the substratum is given a great deal of attention by the perfumer who differentiates between those plants that grow on oak (greenish) and those found on conifers (greyish); in the latter case rightly so, since resins may be included with the lichen, rendering it less desirable for the trade. In all instances the crop is gathered by peasants or by shepherds, as in Jugoslavia, and pressed into large bales for export. The American supply is derived from these sources, importations amounting to a few tons yearly at a cost of from five to seven and one-half cents a pound delivered in New York City. Since the present war has cut off the supply, a few companies, formerly established in France and Holland, have been interested in developing the American market, but the lack of apt collectors willing to work in competition with Europe's cheap labor or even at a somewhat higher rate per pound renders the commercial possibilities somewhat doubtful. Experiments including a number of American species have been carried out with little success except in the case of those traditionally used in the Old World, and of these there are sufficient quantities available in the northern part of the United States and Canada and at the lower altitudes on the higher hills (2000 feet elevation).

b) *Chemical Properties of Essential Oil of Lichens.* Use of dried, pulverized Oak-moss in the perfume industry is restricted, the principal sale being of extracts, essences and "resinoids". Gildermeister and Hoffmann state that the method involves exhausting the lichen by means of a volatile substance and treating with acetone, which removes the resins, waxes and chlorophyl. The extraction by alcohol gives an "extract of Oak-moss" which may be used in this form or may be concentrated in order to obtain a semi-fluid substance. Research on the part of French and German chemists during the last thirty years has revealed much of the chemical nature of the extracts, gums and mucilages produced by these lichens. Gattefossé (75) made a study of the essential oils and alcoholic extracts of all those lichens which were utilized as Oak-moss, and by extraction and distillation obtained data that caused him to conclude that oil of Oak-moss was almost exclusively a compound of phenol called lichenol, considered an isomeric compound of carvacrol. These results were verified by St. Pfau who further expressed the opinion that sparrassol, a metabolic product of the fungus *Sparassis ramosa,* is identical with methyl everninate resulting from the alcoholysis of everninic acid, and is present in proportions of about 2.8% with a characteristic anise seed odor.

Walbaum and Rosenthal questioned the findings of Gattefossé; so they distilled by steam the oil of Oak-moss from *Evernia prunastri.* This, at ordinary temperature, formed an oily crystalline mass of a dark color with a very powerful and agreeable odor. Upon further analysis, it was found that Gattefossé's discovery was founded upon an error, and that not lichenol ($C_{10}H_{14}O$) but orcinol monomethylether is the principal constituent of Oak-moss. This phenol, though not the main odoriferous part of Oak-moss oil, has a pleasant, creosol-like smell, and an ester, β-orcinol methyl carboxylate ($C_{10}H_{12}O_4$), which does not enter into the odor of the lichen oil. In the resinous precipitate, Walbaum and Rosenthal found ethyl everninate generated only during extraction through esterification of the everninic acid ($C_{17}H_{16}O_7$) which they stated occurred in the free state in the lichen, and that during boiling with baryta water it split into orcinol and everninic acid with liberation of carbon dioxide. This acid is closely related to orcinol monomethylether and would be converted into it by the liberation of carbon dioxide. For these reasons Walbaum and Rosenthal felt that the genesis of the principal constituent of the odoriferous substances of Oak-moss had a close connection with the origin of evernic and everninic acids. Recently, Stoll and Schener found in the volatile fraction some compounds which may also have a function in producing this odor, mainly thujone, naphthalene, borneol, camphor, civeole, citronellol, guaniol, vanillin, methylnonylketone and stearic aldehyde.



The multiplicity of types of essences and extracts is due in part to the diversity of substrata on which these lichens grow as well as to the varying mixtures of species offered to the manufacturer in the lot, and the mode of extraction. This is indicated by Hesse (85) who had extracted atranorine and everninic acid from a sample of *Evernia prunastri* on oak but not from samples collected on beech or birch, while a sample from a lime-tree yielded some usnic acid. The whole problem is further complicated by the fact that most constituents of Oak-moss react upon the solvent. Treatment of lichen extracts with alcohol is seldom employed for preparation of essences, since it alters the evernic acid. Thus the lichenol obtained by Gattefossé using this method was everninate of ethyl. Synthesis of everninic acid, divarine and others has been performed in the laboratory but apparently has not been applied on a commercial scale.

Horel states that in the trade, the oil is extracted by means of low boiling solvents, after which it is purified and decolorized, the process yielding 0.2–0.3 kilogram of the raw extract or 20–30 grams of the pure essential oil from different technique of extraction in which 100 grams of the dried lichen yielded 8.5 grams of crude everninic acid.

c) Uses of Essential Oils. The essential oil of Oak-moss or "concrete" is used in soap making in its natural condition, as an impalpable powder or in the form of resinarome. The powder form permits production of soap-balls agreeably scented and at a reasonable price, but unless the manufacturer obtains a perfectly impalpable powder, the soap-balls give the impression of being made from sand. In order to maintain the quality of his product, the soap maker must establish connections with a reliable purveyor. To be sufficiently scented, a soap-ball must have 1 or 1½% by weight of powder to soap. When used for this purpose, Oak-moss improves, strengthens and cheapens lavender-scented products. It is essential in the finer grades of cosmetics in combination with jasmine, tuberose and orange blossom scent. Parry suggests the use of *Cladonia rangiferina* and *Cladonia sylvatica* for purposes of retaining odor, since they are whitish, easily dried and abundant in open healthy places. Some, as *Sticta fuliginosa* and *Sticta sylvatica,* have objectionable odors that are easily recognized by their fishy or methylamine nature. Mention has already been made of the many uses of Iceland Moss in foods and medicines. In the cosmetic field, it serves as a source of glycerol in the soap industry and in the manufacture of cold creams, though it is odorless (166).

Lichens are especially adapted for perfumery, since to the parfumeur the peculiar reciprocity of the components forming the lichen unit and known to the unromantic biologist as "symbiosis" is an example of harmonious action. Therefore, the extract of Oak-moss or Scented-moss "agrees" and "harmonizes" in the happiest manner with a large number of other essences. Its fragrance has been likened to musk-lavender and as such it may be used as a fixative (of the poppy type) blending well with bergamot, citron, acetate of lynalyl and linalol, thus supplying freshness; with neroli, jasmin, rose and cassia, it improves the flavor of these flowers; it gives flexibility to taragon, coriander, portugal, ylang-ylang and vanillin; contributes stability and depth to patchouli, vetyver, coumarin and musk, and elevation to alpha ionene.

It also blends well with synthetic oils, as amyl and isobutyl salicylate and aceto-phenone (141, 169). It is indispensable as the basis of numerous perfumes, such as Chypre, Fern, Heath, and many bouquets called "Fancy", as well as for the Oriental perfumes.

Miscellanea

Gums. The dyeing and paper industries have need for quantities of sizing with which to dress and stiffen silks, print and stain calico and size paper. During the Napoleonic Wars, because of the French monopoly of Senegal Gum, the celebrated English inventor, Lord Dundonald, attempted to introduce lichen mucilage in place of the French product, but there is no evidence that he succeeded in interesting the English market. At Lyons the French appear to have successfully used lichen mucilage as a substitute for gum arabic in the fabrication of dyed materials (82). The problem has been opened more recently by Minford (146) who reports that Iceland Moss and some other lichens may be prepared as light-colored, transparent and high-grade gelatin, isinglass and similar gelatinous products corresponding to those obtained from vegetable products for this purpose.

Injury by Lichens. Lichen injury to artificial structures has been already discussed in relation to damage of stained glass windows of churches (144). Cengia-Sambo (26) reports an interesting incident of stains and incrustations on marble, alabaster and Florentine mosaics resulting from the activity of lichen growths favored by humidity.

Orchardists and silviculturists have long been interested in the probable relationship of lichens to trees. Many sprays, including Bordeaux Mixture, caustic soda and light-boiling tar oils (65), have been recommended for the removal of these "unsightly if not injurious plants" on the grounds that they could hardly be considered as timber preserving plants. Indirectly they may cause an economic loss by serving to shelter the many injurious insects that seek cover and lay eggs. Kaufert notes that the bark of *Populus tremuloides* remains permanently smooth through the presence of a persistent periderm. If injured by fungi, lichens or mechanical injury, the bark may be stimulated to develop rough fissures, since these injuries appear to be associated with rough bark on some aspen stands. In studying the influence of *Usnea* sp. upon trees in South Africa, Phillips concluded that in this case the lichen is definitely detrimental in that its fungal component is parasitic upon tissues external or internal to the cork cambium. Vigorous crowns as well as defective ones may be infected. Since the lichen cannot develop luxuriantly under the conditions obtaining in undisturbed high forests, he recommended that the forest canopy be preserved as a means of inhibiting the rampant growth of this lichen. Wellborn suggested that some leaf spots of the coffee plant may be caused by a lichen. Howbeit, the whole problem of whether lichens injure the trees on which they are fastened cannot be solved, as Fries once remarked, "by mere denial".

APPENDIX. LICHENS EMPLOYED IN DYEING AND MINOR USES[11]

SCIENTIFIC AND COMMON NAMES	REGIONS* WHERE FOUND OR GENERALLY USED	COLORS OBTAINED	USES
Alectoria jubata (L.) Ach. Horsehair lichen	England	pale green, brown-red	woolens (135)
Bacidia muscorum Mudd	Europe	red	woolens (114)
Borrera ashneyi (?)	Chutcheleera, India	flesh-color	woolens (135)
Caloplaca murorum (Hoffm.) Th. Fr.	Sweden	yellow	woolens (90, 114)
Candelaria Mass.	Sweden	yellow	candles (198)
Candelariella vitellina (Ehrh.) Müll. Arg.	Sweden	yellow	woolens (114)
Cetraria aculeata (Schreb.) E. Fr.	Canary Is., Scotland	red-brown	woolens (135)
C. fahlunensis (L.) Schaer. Swedish shield lichen†	Europe	red-brown	woolens (114)
C. glauca (L.) Ach. Pale shield lichen†	Europe	chamois	woolens (90)
C. islandica (L.) Ach. Iceland moss	Iceland	brown (1), yellow (2), red-brown	woolens (135) leather (7)
C. juniperina (L.) Ach. En-mossa, cedar lichen†	Scandinavia	yellow	woolens (135)

* Region given does not represent full range of any one species, most of these are cosmopolitan.

† Common names from Nearing.

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[11] Synonymy brought to date where possible according to Zahlbruckner (232) and Fink (63).

Scientific and Common Names	Regions* Where Found or Generally Used	Colors Obtained	Uses
C. nivalis (L.) Ach. Snow lichen†	Europe	violet	woolens (114)
C. pinastri (Scop.) S. Gray Pine lichen†	Europe	green	woolens (90)
Cladonia coccifera (L.) Willd. British Soldiers	Europe	red-purple	woolens (114)
C. fimbriata (L.) Willd. Trumpet lichen†	Europe	red-purple	woolens (114)
C. gracilis (L.) Willd. Spoon lichen†	Europe	ash-green	woolens (114)
C. rangiferina (L.) Web. Reindeer moss	Europe	iron-red	woolens (114)
C. pyxidata (L.) Hoffm. Goblet lichen†	Europe	ash-green	woolens (114)
Dermatocarpon miniatum (L.) Mann	Europe	ash-green	woolens (114)
Evernia furfuracea (L.) Mann Sprout lichen†	Europe	red-brown	woolens (114)
E. prunastri (L.) Ach. Oak moss	Europe	violet	woolens (114)
E. vulpina (L.) Ach.	Scandinavia	yellow	woolens (198)
Gyrophora cylindrica (L.) Ach. Rock tripe	Iceland	green-brown	woolens (135)
G. deusta (L.) Ach. Rock tripe	Sweden	violet	paint ("Tousch") (119) woolens (114)
G. vellea (L.) Ach. Rock tripe	Sweden	violet	woolens (114)
Haematomma ventosum (L.) Mass. Black Lecanora, Bloody spotted Lecanora	Scotland	red-brown	woolens (135)
Lecanora calcarea (L.) Nyl.	Sweden	red-brown	woolens (2)
L. parella Mass. Light crottle, Crabseye lichen	Gt. Britain France	violet	woolens; Orseille d'Auvergne (135)
Lepraria chlorina (DC.) Ach. Brimstone colored Lepraria	Scandinavia	brown	woolens (135)
L. iolithus (L.) Ach. Viol-massa (Swedish)	Scandinavia	brown	woolens (135)
Lobaria scrobiculata (Scop.) DC.	Scotland England	brown	woolens (135)

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* Region given does not represent full range of any one species, most of these are cosmopolitan.

† Common names from Nearing.

Scientific and Common Names	Regions* Where Found or Generally Used	Colors Obtained	Uses
Aik-raw, Oak rag, Warty leather lichen†			
L. pulmonaria (L.) Hoffm. Oaklung, lungwort, Aikraw, Hazelraw, Hazelcrottle, Rage, Stane Raws	Scandinavia Gt. Britain	orange, brown	wool; (+indigo = lasting black) (135)
Mycoblastus sanguinarius (L.) Norm. Red fruited lecidea	Scotland	red-purple	woolens (135)
Nephroma parile Ach. Chocolate colored Nephroma, Powdery Swiss lichen†	Scotland	blue	woolens (135)
Ochrolechia tartarea (L.) Mass. Crotal, Crottle, Cockur, Corcir, Korkir	Scotland and adjacent islands Sweden, France	purple-crimson, blue	Cudbear (19), tincture of cudbear (187), (+logwood = red-purple) litmus
Parmelia acetabulum Duby	North Ireland	brown, orange	wool, homespuns, Harris tweed (135)
P. caperata (Hoffm.) Ach. Stone crottle, Arcel, Wrinkled shield lichen†	Isle of Man	brown-orange, lemon, yellow	woolens (135)
P. centrifuga (L.) Ach. Ring lichen†	Gt. Britain	red-brown	woolens (90)
P. conspersa (Ehrh.) Ach. Sprinkled parmelia, Boulder lichen†	England	red-brown	woolens (135)
P. Kamtschadalis (?)	India	pale rose	to print and perfume calico cloth (143)
P. olivacea (L.) Ach. Bronze shield lichen†	Gt. Britain	brown	woolens (143)
P. omphalodes (L.) Ach. Smoky shield lichen,† Black crottle, Corks, Cockur, Crostal	Scandinavia Scotland Iceland	purple, crimson	woolens (135)
P. physodes (L.) Ach. Dark crottle, Puffed shield lichen†	Scotland Scandinavia	brown	woolens (19)
P. saxatilis (L.) Ach. Stane raw, Scrottye, Stenlaf (Swedish)	Scandinavia Gt. Britain	dirty orange, yellow, red-brown	woolens (19) wool (176)

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* Region given does not represent full range of any one species, most of these are cosmopolitan.

† Common names from Nearing.

Scientific and Common Names	Regions* Where Found or Generally Used	Colors Obtained	Uses
P. stygia (L.) Ach. Midnight lichen†	Gt. Britain	brown	woolens (143)
Peltigera canina (L.) Willd. Dog lichen†	Europe	iron-red	woolens (114)
Pertusaria pseudocorallina (Sw.) Arn. Westring's Isidium	Norway Sweden	red-purple	woolens (135)
P. corallina (L.) Arn. White crottle	Scotland	red-purple	woolens (135)
Physcia pulverulenta (Schreb.) Nyl. Mealy Blister lichen†	Europe	yellow	woolens (143)
Ramalina calicaris (L.) Röhling Common Twig lichen†	Europe	yellow-red	woolens (114)
R. cuspidata (Ach.) Nyl.	Europe	light brown	woolens (176)
R. farinacea (L.) Ach. Mealy Ramalina	England	light brown	woolens (135)
R. fraxinea (L.) Ach. Ash Twig lichen†	England	gray-white	woolens (114)
R. scopulorum Ach. Ivory-like ramalina	Scotland	red-brown	woolens (135)
Rhizocarpon geographicum f. *atrovirens* (L.) Mass. Map lichen	Scandinavia	brown	woolens (135)
Roccella fuciformis (L.) Lam. and DC. "Lima" weed, "Angola" weed Orcella, Orcigilia; Orchil	France England	purple-crimson, red-yellow	Orchil, litmus; wool, silks, feathers, carpet yarns; and staining wood and marble (49, 91)
R. Montagnei Bél. Orcella weed	Germany, Italy	(+ other dyes = brown, maroons, claret)	white wine; and with indigo (140, 108)
R. phycopsis Ach. Archil	Burma	blue	for British broadcloth; tincture of alcohol for thermometers (143)
R. tinctoria Lam. and DC. Orseille (Valparaiso Weed)	South France	purple-crimson	"bluing" agent

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* Region given does not represent full range of any one species, most of these are cosmopolitan.

† Common names from Nearing.

Scientific and Common Names	Regions* Where Found or Generally Used	Colors Obtained	Uses
Solorina crocea (L.) Ach. Saffron-yellow Solorina	Scotland	yellow	woolens (135)
Stereocaulon paschale (L.) Hoffm. Eastern lichen†	Europe	ash-green	woolens (114)
Sticta aurata Ach. Rose & gold lichen†, Lungwort Hazelraw, Hazel crottle	Gt. Britain Scandinavia	saffron, brown	woolens (135)
S. crocata (L.) Ach. Rags, Oakrag, Gold Edge licken†	Scandinavia Gt. Britain	gamboge, brown	woolens (135)
Teloschistes parietinus (L.) Norm. Wäg-laf (Swedish)	England Sweden	yellow	woolens (135) Easter eggs (135)
T. flavicans (Swartz) Norm. Yellow Borrera	Germany	gamboge, yellow	woolens (135)
Umbilicaria pustulata (L.) Hoffm. Blistered umbilicaria	Norway Germany	red, purple, brown	woolens (119) black paint (43)
Urceolaria calcarea Sommerf. Corkir, Limestone Urceolaria	Gt. Britain Western Islands	red-crimson	woolens (135) cudbear (49)
U. cinerea Sommerf. Greyish Urceolaria	England	red-crimson	woolens (135)
U. scruposa (Schreb.) Ach. Rock Urceolaria	England	red-brown	woolens (135, 143)
Usnea barbata (L.) Wigg. Bearded Usnea, Old Man's Beard†	S. America Pennsylvania, U.S.	orange-red	woolens (19)
U. florida (L.) Web. Flowering Usnea	Europe	green-yellow, red-brown	woolens (135)
Usnea plicata (L.) Wigg. Plaited Usnea	Europe	green & yellow	woolens (114)
Usnea sulphurea Th. Fr.	Falkland Is.	brown	woolens (3)
Variolaria orcina Ach.	France	violet	woolens, orchil (140)
Xanthoria candelaria (Ach.) Arn. Ljas-mässa (Swedish) (=*Teloschistes candelarius*. (L.) Fink)	Sweden	yellow	woolens (143)

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* Region given does not represent full range of any one species, most of these are cosmopolitan.

† Common names from Nearing.

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ROOT-ROTS OF CERTAIN NON-CEREAL CROPS[1]

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INTRODUCTION

In a recent publication Simmonds has outlined some of the trends which have appeared in research on root-rots of cereals (286)[2]. The present paper aims to give the status of root-rots of certain non-cereal crops, though no claim is made for an exhaustive review of such rots.

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[2] Reference numbers are given in the text along with an author's name only when there is more than one citation for the author in the bibliography.

That the bulk of the literature on root-rots is of comparatively recent origin is not to be wondered at, since it was only natural that the early pathologists should have investigated first the more obvious diseases, namely, those attacking the above-ground parts, these parts being more apparent and forcing themselves on the attention of those interested in plant diseases. Accordingly, the diseases due to parasitism below ground, that is, root-rots, were left, for the most part, till a later date. One of the several exceptions in this connection was the root-rot of cotton, when Pammell in 1888 reported that the disease which had been killing cotton in Texas was due to a fungus which invaded and killed the roots, and suggested that it could be controlled by proper rotation of crops. The progress made with cotton root-rot investigations in the next 49 years is shown in one list of 157 papers, of which 140 were published during the past 25 years (292). In checking over 619 citations in six papers on root-rots of cotton (292), tobacco (204), sugar cane (247), strawberry (17) and cereals (104, 286), it was noted that 573 or 92.6% also were dated in the last quarter century.

Though most root-rots are general in distribution and few crops completely escape their attack, certain of them may have rather limited distribution. For instance, the destructive one of cotton caused by *Phymatotrichum omnivorum* is confined to the southwestern United States, especially Texas, and adjoining Mexico. Rhododendron wilt and root-rot caused by *Phytophthora cambivora* and the black root-rot of apple caused by *Xylaria mali* have been reported only from the United States. On the other hand, *Armillaria* root-rot of fruit and forest trees and small fruits, and the root-rots of sugar cane and strawberries are present in practically every country where these crops are grown.

The above-ground symptoms of root-rots consist, for the most part, of wilting, yellowing, stunting and often death of affected plants. However, definite diagnosis cannot be made without recourse to examination of roots. Macroscopic examination permits diagnosis of a few common rots characterized by special symptoms, for example, *Armillaria* and *Clitocybe* mushroom root-rots, especially if sporophores are present; red-stele root-rot of strawberries, because of the colour of the stele; black root-rot of tobacco, on the basis of colour; cotton root-rot, especially if spore mats or sclerotia are present; and *Pythium* root-rots of the water-soaked type. But it is exceedingly difficult if not impossible, without recourse to microscopic examination and isolation, to diagnose many others, since rotted roots, irrespective of causal organism or organisms, have much the same appearance. This is especially true of complex rots where several fungi may be associated with the disease.

As the following résumé points out, not only are many different crops attacked, but the fungi involved are numerous and varied in character, especially as to degree of parasitism. Some are highly specialized parasites that might be classed as "soil invaders", while others, less specialized, more correctly belong to the category of common "soil inhabitants", using these terms in the sense adopted by Garrett (103). Our knowledge of the interrelationships between the hosts and the fungi involved in producing root-rot on these hosts is in far too many instances scanty and incomplete. This is not surprising when it is realized that roots grow in an inert environment of a most complex physical and chemical nature, inhabited by countless numbers and species of living organisms in a dynamic equilibrium. It is this living environment to which roots are subjected that makes the study of root-rots most difficult and involved, since it becomes necessary to evaluate not only the direct host-parasite relationship but also the many direct and indirect effects that changing environment exerts on the biology of the soil in general and the indirect effects these changes in turn exert on the host-parasite relationship. This is particularly true of the complex root-rots where several parasites are implicated.

ROOT-ROTS OF VARIOUS CROPS

Woody perennials. "Mushroom root-rot", caused by *Armillaria mellea* (Vahl.) Fr., is associated mainly with forest and shade trees (43, 127, 128), fruit trees including citrus, small fruits, and other crops, as, for instance, potato. In Europe *A. mellea* attacks primarily forest and shade trees, while in America it affects principally fruit trees and small fruits, and to a less extent forest and shade trees. In Australia in 1910 (161) and in Washington in 1912 (12) it was the cause of a field rot of potatoes. Thus it is evident that the host range is extensive and the disease practically world-wide.

Another "mushroom fungus" capable of causing root-rot of pear, peach, apple, tung and other trees is *Clitocybe tabescens* (Scop.) Bres., though its general distribution is by no means so extensive as that of *A. mellea.* This rot was reported first on apple in Oklahoma (339), and it has since been found in Florida on 139 species of plants, including a wide variety of fruit, forest and ornamental trees, shrubs and vines (255).

Both *Armillaria* and *Clitocybe* occur mainly on newly cleared land, infection coming from diseased root particles remaining in the ground after the land is cleared. Although in the absence of sporophores these two root-rots may be difficult to distinguish, since the rhizomorphs of both fungi are rather similar, the mycelial growth characteristics are quite different, the mycelium of *A. mellea* and *A. fuscipes* being generally phosphorescent in the dark, a character absent from many cultures of *C. tabescens* (254). Though Rhoads was unable to obtain infection with pure cultures of *C. tabescens* (254), Plakidas has recently been successful in producing it on pear and tung trees by pure culture inoculation (239).

Poole has reported a "mushroom root-rot" on Lucretia dewberry caused by *Collybia dryophila* Fr.

In Europe a root-rot of fruit trees, especially apple and vine, caused by *Rosellinia necatrix* (Hartig) Berlese, has been prevalent many years, having been first reported in 1883 under the name of *Dematophora necatrix* Hartig (129). Later Berlese transferred this fungus to the genus *Rosellinia.* However, it was not till 1934 that the disease was reported in America as causing serious losses to apple trees in California (301). It was pointed out at that time that this rot may be distinguished from those caused by *A. mellea, C. tabescens* and *Xylaria mali* by the absence of well defined rhizomorphs or stromata and the presence of profuse white cottony mycelium adjacent to affected roots. In 1937 this disease was again reported and its genetic relationship with *R. necatrix* confirmed (124). Another species was reported in California on apple orchards and tentatively identified as *R. aquila* (Fr.) De Not. Fawcett cites *R. pepo* Pat. and *R. bunodes* (B. et Br.) Sacc. as root parasites of citrus trees and cacao (87). *R. necatrix* has been reported recently as causing a root-rot of alfalfa in California (125).

Another white root-rot of apple and other fruit trees first reported in 1902 (327) is caused by *Corticium galactinum* (Fr.) Burt. In 1909 this rot was present in various parts of the Ozark Mountains and was able to spread from oaks to fruit trees when the latter were planted on cleared land (328). More recently it has appeared in

Virginia, Maryland, Tennessee, Delaware and Indiana, and its development is "usually so rapid and complete that the presence of the disease in the roots is manifest only in the top by the sudden death of the whole tree in contrast to the much slower acting root rots, as for instance *X. mali* which may be manifested in the top by weak limbs on the side of the tree above the rotted roots" (55). Dewberry, blackberry, Japanese wineberry, dogwood and sumac have been found infected when growing near affected apple trees.

The black root-rot of apple caused by *Xylaria mali* (Ellis & Ex.) Fromme is confined to the more southern States, Virginia, Kentucky, North Carolina, South Carolina, Tennessee, West Virginia, Illinois and Indiana. According to Thomas, *X. polymorpha* (Pers.) Grev. occurs commonly on apple in New York State, and *X. mali* is unknown there (299). The presence of the stromata of *Xylaria* and their attachment to the main trunk and roots, as well as the characteristic black incrustation on the roots, and the brittle, punky character of the invaded wood are certain diagnostic features of this disease. In 1938 Fromme and Schneiderhan demonstrated that stocks became infected with *X. mali* when exposed to infested soil or diseased roots, and they successfully infected trees with pure cultures of the fungus (93). These studies also indicated no resistance in 45 clones of *Malus* exposed to natural infection or in any one of 12 clonal stocks and 11 seedling stocks inoculated with pure cultures.

In 1936 a root-rot of nursery and orchard apple trees caused by *Sclerotium rolfsii* Sacc. was reported (54, 319).

Species of *Phytophthora* are responsible for root-rots of chestnut, beech, cypress, citrus and pine seedlings. Day (65) found *P. cambivora* (Petri) Buis. to be the cause of ink disease of sweet chestnut (*Castanea sativa*), and also associated with a root-rot of beech. Furthermore, *P. cinnamomi* Rand and *P. syringae* Klebahn were isolated from affected roots of chestnut and beech, respectively. Beech is less susceptible than chestnut. Inoculation studies (66) demonstrated that *Castanea sativa, C. crenata* and beech were all susceptible to *P. cambivora, P. syringae* and *P. cinnamomi.* Regarding a root- and collar-rot of *Pinus resinosa* seedlings caused by a species of *Phytophthora* closely resembling *P. cinnamomi,* inoculations with the fungus produced typical effects (152). In this connection it is interesting to note that though species of *Pestalozzia, Sphaeropsis, Sclerotium* and *Rhizoctonia* were associated with *P.*

cinnamomi, none of them produced the typical root-rot in artificial inoculation, though they were capable of producing resiniferous lesions. In 1940 a destructive root- and crown-rot of *Chamaecyparis* was caused by an apparently new species, *P. lateralis* Milb. (216). Plants inoculated with this fungus died within two to six months and the same fungus was re-isolated. According to Petri (236), the most common forms of root-rot of citrus in Sicily are caused by *P. parasitica* Dastur. and *P. citrophthora* (Sm. & Sm.) Leonian, though several other fungi are also associated. *P. parasitica* is, however, the more prevalent. A wilt and root-rot of young rhododendrons, caused by *P. cambivora,* has been described by White (338) and reported also on *Cinnamomum burmanni* (244), *Persea americana* (314, 315, 316, 329), *Pinus resinosa* (152), *Ananas comosus* (195, 214, 280, 281, 282, 284), *Castanea sativa* (217, 235), *Castanea dentata, C. molissima, C. pumila, C. crenata* and *Quercus prinus* (217), *Erica* sp. (24, 229, 317), *Dicranopteris emarginata* (214), *Juglans* sp. (317), *Picea canadensis* and *Quercus rubra* (61) and *Allium cepa* (281, 282).

Other fungi associated with root-rot of citrus in various countries are *Armillaria mellea* (147, 148), *Sclerotinia libertiana* Fckl. (88), *Rosellinia pepo, R. bunodes* (87) and various species of *Fusarium* (89).

Tobacco. Tobacco is attacked by two root-rots, black root-rot caused by *Thielaviopsis basicola* (Berk.) Ferraris (231, 275) and brown root-rot, the cause of which is not known. Since both rots cause stunting and yellowing, examination of roots is necessary for diagnosis. Black root-rot may cause considerable damage to the crop, especially in cool, wet, alkaline soils. Killebrew in 1884 was possibly the first to note and record that "the plants so affected (black root) do not die but after standing comparatively still for a long time revive late in the season but do not make a good quality of tobacco". This characteristic sequence of attack and "standing still" in the early part of the summer, followed by partial recovery, is common with black root-rot. The fact that *T. basicola* requires cool, moist conditions for best growth explains why more severe damage is caused in cool, wet, backward springs and why affected plants outgrow to a large extent the effect of the attack when the warm, dry conditions of summer prevail. Recently, Rawlings reported on three distinct physiologic races of *T. basicola,* one of which was strongly pathogenic to tobacco, groundnuts and watermelon, but less so to cotton and *Primula obconica;* another damaged watermelons severely, groundnuts less and failed to attack tobacco; while the third was only weakly parasitic to cotton, groundnuts and watermelon, and unable to infect tobacco or *P. obconica*. *T. basicola* has also been found associated with root-rots on bean, soybean, red clover, cotton, cyclamen, tomato, flax, peanuts, violets, ginseng, cowpeas, parsnips, poinsettia and lupines. Rawlings states that this fungus has been found on 120 species in 30 families.

Though there has been considerable research on brown root-rot of tobacco since 1915, when it was first reported (155), the cause has still to be ascertained. Various agencies have been suggested, namely, parasitic organisms (204), toxic substances in the soil (15, 71), and nutritional disorders (79). The outstanding characteristic of this disease on which there is general agreement is that previous crops of timothy and corn especially favour it. It is considered that such crop effects may be due to toxic substances (274, 31, 51) or depletion of certain substances required by the plants that follow. Infusions of timothy have produced injurious effects on roots of tobacco (71), suggesting that brown root-rot may be only the expression of injurious effects of preceding crops, and in other investigations on infusions of timothy, red clover and tobacco the nitrogen fractions of different infusions were different (78). Later, Eisenmenger demonstrated that timothy and corn decomposed very slowly, whereas tobacco decomposed very rapidly (79). He was able to divide the plants into two groups, the one group—corn, timothy, *etc*., unfavourable to precede tobacco—showed a high percentage of total nitrogen, a high percentage of total soluble nitrogen, the highest percentage of protein, a low percentage of amides, a high percentage of ammonia, and the lowest percentage of nitrate nitrogen. The other group—favourable as crops to precede tobacco—showed lower percentages of each of these fractions except nitrates. The nitrates in this group were higher. Eisenmenger suggests that plants containing high lignin, high pentosans, high carbon-nitrogen ratios and a subsequent low tendency to protein decomposition in soil may be suspected of being undesirable for tobacco rotation.

Beaumont has also shown a relationship between brown root-rot and decomposition of organic matter. He found that ammonium compounds, amino acids and certain amides were toxic to tobacco and caused symptoms very similar to those of brown root-rot. He suggested that the disease is indirectly caused by the unoxidized forms of nitrogen that result from decomposition of organic matter, and considered that the root-rotting is due to high nitrogen concentration in the roots, or a narrowed carbon-nitrogen ratio brought about by the rapid absorption of basic nitrogen which makes the roots very susceptible to decay organisms in the soil. He found root-rot most severe under conditions not favourable to nitrification.

More recently it was suggested that *Sclerotium bataticola* Taub. may be the cause of brown root-rot, and it was shown that the cultural habits of *S. bataticola* are in agreement with the limiting factors as related to root-rot under field conditions (204). The authors also were able to produce a condition very closely resembling brown root-rot by inoculation under greenhouse conditions in the presence of a source of carbohydrate, that is, filter paper. In this connection a temperature of 45° C. for 24 hours or 48° C. for 10 to 12 hours completely destroyed the disease-producing agency, but *Rhizoctonia* (*Sclerotium*) *bataticola* (Taub.) Butler was able to withstand 45° C. more than 48 hours, thus raising a doubt as to *S. bataticola* being the causal agent (157). Johnson has also pointed out that careful microscopical examination of freehand and paraffin sections "failed to show either frequent or extensive invasion of the affected cells by fungi of any sort", a condition not in keeping with the theory of parasitic attack. He concludes: "the failure to find a microscopically visible organism in or between the diseased cells lends support to the idea that a typical parasite is not involved". At the same time he says: "it does not disprove the possibility that organisms confined largely to the exterior of the roots or harboured only in the soil itself may excrete toxic substances that are necrotically injurious to the roots" (157).

Cotton. In the southwestern United States *Phymatotrichum* root-rot of cotton is widespread and serious. In addition to cotton this fungus attacks some 1700 species of plants (297), a fact which must be taken into consideration from the standpoint of crop rotation and control measures. Because of this fact, as well as the nature of the fungus itself which produces sclerotia in abundance, this rot has been most difficult to control, and has received more attention by research workers than possibly any other root-rot to date. The pertinent literature, most of which deals with various pathological and agronomical phases of the disease, has recently been reviewed (292, 250). In broad outline these papers have dealt with symptoms, the fungus responsible, rate and manner of spread, influence of environment, host range, varietal susceptibility, reaction of soil and control, including crop rotation, fallow, cultivation, soil amendments, especially with manure, organic materials and fertilizers. More recent papers deal with control and its microbiological aspects (62, 220). *Phymatotrichum* root-rot is of special interest in that it seldom produces stunting or dwarfing, as is the case with many root-rots, but rather causes yellowing and wilting of the leaves, followed rapidly by death in most cases. In addition, this rot may be diagnosed by the presence of spore mats on the surface of the ground around affected plants. These mats, which vary from a few inches to a foot or more in diameter, are white at first but later turn a tan colour. In addition to spore mats this fungus produces sclerotia in abundance, under some conditions, and their presence is helpful for diagnostic purposes. In the Punjab of India both American and indigenous cotton is subject to a root-rot in which *Rhizoctonia solani* and *R. bataticola* are involved. This disease has been studied from many angles, including physical and chemical factors, chemical composition of healthy and diseased plants, temperature and moisture relationships and the effects of intercropping (321–326, 198).

Sugar beets. Several fungi are associated with root-rots of sugar beets. Wherever they are grown, black-root is of considerable importance. In Europe especially this root-rot has caused serious losses and has been the subject of many investigations. Black-root is primarily a seedling disease, and as the disease may be seed-borne, seed treatment is generally recommended. Ceresan has given satisfaction in most cases. Five different fungi are associated with the condition, namely, *Phoma betae* (Oud.) Fr., *Rhizoctonia solani* Kühn, *Pythium debaryanum* Hesse, *Aphanomyces cochlioides* Drech. and *Fusarium* sp. Of these it is generally agreed that *P. betae* is the most important, as regards both distribution and degree of parasitism. Campbell in Washington reports that of 31 different fungi isolated from sugar beets and tested for pathogenicity, only *P. betae* gave positive results. In Germany sugar beets are subject to root-rot or blackleg caused by *P. betae*, *P.*

debaryanum and *Aphanomyces levis* Edson non DBy, *P. betae* being more common and important (178, 232). In Sweden *P. debaryanum* is important (11).

In addition to being associated with other fungi in causing black-root of sugar beet seedlings, *R. solani* is also capable of producing a dry rot of sugar beets at all stages of development. A study of 78 isolates of *Rhizoctonia* from sugar beet and potatoes gave evidence in support of the claim that the strains from potato stems or tubers are unable to infect sugar beet, indicating that the sugar beet strains may be distinct pathogenically from those attacking potato (190, 192). On the other hand, there are cases where *Rhizoctonia* root-rot on sugar beet was much more severe when sugar beets or potatoes preceded sugar beets than when sugar beets followed maize or small grains (barley) (37, 206, 207). Buchholtz reported that 50% of the sugar beets on the part of a field previously planted to potatoes became infected with *Rhizoctonia,* while only 1.6% became infected on that part of the same field which had been cropped to barley the previous season. In this connection, though 64 isolates from potato stems or tubers were unable to infect sugar beets, three isolated from potato stolons were capable of causing appreciable decay of sugar-beet roots, while nine isolates produced slight decay and thirteen were non-pathogenic (192). Field surveys in 1936, 1937 and 1938 indicated that where sugar beets followed sugar beets, root-rot was more severe than where sugar beets followed potatoes. In fact, sugar beets following potatoes were relatively free from root-rot. Nevertheless, in Iowa and elsewhere root-rot has been correlated with preceding crops of potatoes, and the fact that certain strains of *R. solani* may attack potatoes, as shown by Le Clerg, would explain such an association. In addition, there is the possibility that other strains of *R. solani,* though unable to parasitize potato stolons or tubers, may under favourable conditions "colonize" as saprophytes on or in close proximity to potato roots and thus be ready to attack sugar beets the following season. Sugar beets, as well as certain other field crops, are subject to attack by another species, *R. crocorum* (P.) ex Fr. (*Helicobasidium purpureum*), known as the violet, red or root killer root-rot.

Leach (183) considers the most important root-rots of sugar beets in California to be southern rot caused by *Sclerotium rolfsii*

Sacc. and dry rot canker caused by *Rhizoctonia solani,* though a wet rot caused by *Phytophthora* sp. is also of some importance. In Utah *Phytophthora drechsleri* Tucker was destructive mainly in the heavier, poorly drained soils (307). In addition to California and Utah, it has been found also in Idaho and Colorado. In 1939 Greis reported that *Macrosporium cladosporioides,* hitherto known only as a saprophyte associated with sugar beets, was in reality a joint agent with *Pythium debaryanum* and *Alternaria tenuis* in causing a typical root-rot of sugar beets in Germany.

Peas, vetches, clovers. Several organisms are recognized as causing root-rot of peas, vetches and sweet clover. *Aphanomyces euteiches* Drech. is of prime importance, with species of *Rhizoctonia* and *Pythium* playing a secondary rôle (74). Another root-rot of peas in Canada is due to *Fusarium vasinfectum* var. *pisi* Atk. and *P. debaryanum* in association (290, 291). *Fusarium* alone produces a browning and *Pythium* a blackening of the tissue, whereas both together produce typical root-rot or blight. Weimer (334) considers that root-rot of peas in southern United States is due primarily to *A. euteiches* with which are associated *P. graminicola* Subr., *P. irregulare* Buis., *Rhizoctonia* sp., *Fusarium* sp. and bacteria. In Tasmania, too, a root-rot of peas is caused by *A. euteiches* (105). Though species of *Fusarium* and *Pythium* were isolated from diseased roots as well as *A. euteiches,* inoculations indicated that neither of these fungi was capable of producing the rot. In New Jersey a root-rot of peas caused by *A. euteiches* also attacked sweet pea, hairy vetch, cowpea and sweet clover (119, 120). *Fusarium coeruleum* (Lib) Sacc. (126), *F. martii* var. *pisi* App. and Wr., *R. solani, Ascochyta pinodella* L. K. Jones and *Mycosphaerella pinodes* (Berk. & Blox.) Stone (331) have also been associated with root-rot of peas.

In Wisconsin (331), the Middle Atlantic (74) and the Southern (334) States, as well as in Tasmania, *A. euteiches* is considered to be the most important organism in the pea root-rot complex. However, in New York State *Fusarium solani* (Mart.) v. *martii* (App. et Wr.) Wr. f. 2 Sny. was the most important organism (252*a*). In a comprehensive study over a ten-year period (1931–41), *F. solani* v. *martii* f. 2 was an active parasite under all conditions investigated, whereas *A. euteiches* was of little consequence under dry conditions (252*a*). The organisms associated with pea root-rot ranked in the following order of importance: *F. solani* v. *martii* f. 2, *A. euteiches, P. ultimum, R. solani* and *Ascochyta pinodella* (252*a*). Jones has recorded a wilt and root-rot caused by *F. vasinfectum* v. *pisi* as being very destructive in the United States (162). This disease differs from the other typical root-rots in that it is primarily a vascular disease resulting in wilt, though roots are also attacked. Buisman considers that *P. irregulare* is the cause of a root-rot of peas in Holland.

R. solani is also capable of producing a serious root-rot of sweet clover, as reported recently in Manitoba, Canada, and Minnesota. All seedlings in inoculated plots were killed under certain conditions, and *R. solani* was isolated most commonly in the early stages, but species of *Fusarium* predominated in more advanced stages, thus indicating that *R. solani* was primarily responsible (46).

Phytophthora cactorum (Leb. & Conn.) Schroet. was shown in 1940 as the cause of a destructive root-rot of sweet clover in Alberta (59). The same disease had been reported earlier as being caused by *Phytophthora megasperma* Drechs. (163), though the two authors now agree that the fungus responsible for root-rot of sweet clover in the United States is *P. cactorum* and not *P. megasperma*. *P. cactorum* is also responsible for a black root-rot on hops in New Zealand (32).

Sugar cane, corn, pineapple. *Pythium arrhenomanes* Drechsler is a strong parasite against the roots of sugar cane in practically every sugar-producing country. Carpenter in Hawaii was the first to establish this. While it is the principal agent in the United States, 12 other species of *Pythium,* including *P. dissotocum* Drech. and *P. graminicolum,* and other fungi are associated with the rot (247). In Coimbatore, a root-rot of sugar cane was caused by *P. debaryanum* (243). *Rhizoctonia solani* and *R. palida* Matz. have also been reported as causal agents, especially in Puerto Rico (205), Barbados (28) and Louisiana (77), but they are not considered so important as *P. arrhenomanes.* In addition to sugar cane, *P. arrhenomanes* and other species of *Pythium* attack the roots of field corn and milo sorghum (80, 154, 258), causing a serious rot under certain field conditions. It was found that in most cases *P. debaryanum* was the first parasite attacking corn roots, followed by *P. graminicola* when the temperature became higher. At the same time *Gibberella saubinettii* (Durieu & Mont.)

Sacc. and *R. solani* became active, and as the season advanced, *Helminthosporium sativum* P.K. & B. and *Fusarium* spp. produced further necrotic lesions, thus suggesting that corn root-rot is of a complex and progressive type (140, 141). From a root-and-stalk-rot complex of sorghum, *Sclerotium bataticola* and species of *Fusarium, Helminthosporium* and *Pythium* (not *arrhenomanes*) were isolated, and inoculation tests indicated that *Pythium* sp. and *S. bataticola* were capable of causing the seedling blight stage of this complex (145). In Ontario members of the genus *Helminthosporium,* and to a lesser degree *P. debaryanum,* were associated with *P. arrhenomanes* as causal factors of root-rot of field corn (258).

In connection with root-rots of pineapple, nine species of *Nematosporangium,* eleven of *Pythium, Pseudopythium phytophthora, Phytophthora meadii* and *P. melongenae* were more or less pathogenic to smooth Cayenne roots in Hawaii (282, 283). Of these, *N. rhizophthoron, N. polyandron, Pythium splendens* and *P. diamesan* produced more severe damage than the others. However, Rands and Dopp (246), after comparing the above nine forms of *Nematosporangium* with *P. arrhenomanes* from corn and sugar cane, concluded that all nine forms should be referred to *P. arrhenomanes.* Sideris has also described a new species, *Rhizidiocystis ananasi,* as a strict parasite of root hairs of pineapple (278). In Queensland, Australia, Lewcock reports a root-rot of pineapple caused by *Phytophthora cinnamomi.* Johnson found that *P. arrhenomanes* (*N. rhizophthoron*) and species of *Fusarium, Verticillium* and *Rhizoctonia* were associated with root-rots of pineapple in Hawaii. He also reports on *Rhizidiocystis ananasi* in this connection.

Flax. In connection with a root-rot of flax, *Pythium megalacanthum* de Bary was parasitic in the absence of *Asterocystis radicis* de Wildeman (40) which had been reported previously as the cause (200). Buisman suggests that "flax-fire" (scorch) or wilt disease of flax is not a simple disease, but that several fungi may be associated with it in addition to *A. radicis* and *P. megalacanthum.* Diddens verified these findings when she demonstrated that *P. megalacanthum* was responsible for flax scorch. She also found that *A. radicis, Thielaviopsis basicola* and various Phycomycetes (probably *Pythium* spp.) were present in diseased roots, though to a much less extent than was *P. megalacanthum.* In this connection Boyle has shown certain characteristics of varietal resistance of flax to wilt and root-parasitic fungi with which *T. basicola, P. megalacanthum* and *Rhizoctonia* sp. are associated in North Dakota.

Soya beans, broad beans. A root-rot of soya beans caused by *Pythium debaryanum* has been reported in North Carolina (193), and one of broad beans (*Vicia faba*) in Melbourne district, Australia, caused by *Pythium fabae* (45).

Ginseng. In North America ginseng is subject to at least two root troubles, a "rust" and a "disappearing soft rot", with which species of *Ramularia* have been associated (16, 136, 346). Though Hildebrand was able to prove that *R. panacicola* Zins. as well as two new species, *R. mors-panacis* Hildebrand and *R. robusta* Hildebrand, were capable of producing the rot, he was unable to do so respecting the rust form. He suggests, however, that it is probably caused by representatives of *Ramularia,* since members of this genus were commonly isolated from rusted roots. From Korea come reports of two rots, a red rot and an amber-coloured rot, both of bacterial origin (222).

Strawberries, raspberries. Strawberries are subject to two important root-rots, the red core or red stele root-rot, as found in England, Scotland and America, and the black lesion root-rot complex, as found in England, Canada, France, Holland, Australia, Africa and the United States. In 1934 Berkeley (17) summarized the situation as it existed at that time, and pointed out that the decline of strawberries in many countries was hardly to be attributed to any one cause, though root-rots must be considered as one of the factors.

The red stele or Lanarkshire disease, undoubtedly the more serious, was first ascribed to a species of *Phytophthora* though without proof of its pathogenicity (2–4). In 1940 Hickman, working in England, isolated the red stele *Phytophthora,* proved its pathogenicity and named it a new species, *P. Fragariae* (134). The disease was first reported in the United States in Illinois in 1935, and since then has been found in Delaware, New York, New Jersey, Maryland, California, Virginia and Michigan. Its outstanding symptom is the red coloration of the stele in affected roots, whence it derives its name. Oospores of the fungus are generally to be found in the discolored stele. Another characteristic is the lack of fibrous roots, which has given rise to the term "rat tail roots", so commonly associated with this disease.

The black lesion or black root type of root-rot, though more widespread, is not so destructive as the red stele disease. Though this type of root-rot has been reported in most countries where strawberries are grown, and though the cause has been ascribed to various factors, such as winter injury, spring frosts and unfavourable soil, it is generally agreed that certain parasitic fungi play a leading rôle in its production. However, the unusual feature is that no single fungus is constantly associated with it, but rather several organisms. Moreover, though several of the fungi appear to be constantly associated with black root-rot wherever it is found, there is considerable variation in the "associated groups of fungi". Hence, this is regarded as a root-rot complex. For instance, in England *Coniothyrium Fuckelii* Sacc., *Fusarium orthoceras* App. & Wr., *Cylindrocarpon radicicola* Wr., *Pachybasium candidum* Sacc. and *Hainesia Lythri* (Desm.) v. Höhn were capable of producing the lesion type (20), and previously *C. Fuckelii* and *H. Lythri* were found responsible for it in Michigan (293). In Ontario species of *Pythium, Ramularia, Alternaria, Verticillium, Cylindrocladium, Rhizoctonia* and *Asterocystis,* plasmodiophoraceous fungi and the phycomycetous mycorrhizal fungus which has been found in the roots of many plants, are all associated with the lesion type of root-rot (313). Hildebrand, also working in Ontario, has reported that microscopic examination showed the presence of "endophytic mycorrhizal fungus", two forms of *Rhizoctonia, Asterocystis, Pythium* spp., *Ramularia* and nematodes, thus indicating their association with black root (135). In Utah isolations from black lesion root-rot most commonly gave *Fusarium orthoceras* and species of *Rhizoctonia, Cylindrocarpon, Hainesia* and *Coniothyrium* (257). Inoculations showed *F. orthoceras, R. solani* and *Cylindrocarpon obtusisporium* capable of inducing the rot. *Olpidium brassicae* and the phycomycetous mycorrhizal fungus were also present in the cortical layers of affected roots.

In addition to the above, species of *Fusarium, Rhizoctonia, Actinomyces, Pythium* and *Coniothyrium* and other fungi have been reported in various countries as being associated with a root-rot condition of strawberries. Though *R. solani* was associated with root-rot of strawberries in Michigan in 1924 (56) and in Kenya in 1926 (208) it remained for Zeller to prove this relationship by inoculation. Confirming evidence has been reported from Southern Rhodesia (146), Canada (135, 313), Florida (35), Tennessee (276), California (300), New Jersey (202), Australia (1) and Louisiana (238).

According to Berkeley (18), raspberries are subject to a root-rot complex similar to that affecting strawberries. He found that *Coniothyrium Fuckelii, Cylindrocarpon radicicola, Fusarium orthoceras* and *Rhizoctonia solani,* species of *Pythium, Cylindrocladium* and *Rhizoctonia* (orchid type), and nematodes were associated with it. These same organisms have been connected with root-rot of strawberries, and Berkeley found both strawberries and raspberries afflicted with the complex in certain fields of British Columbia.

Vegetables. So far as vegetables are concerned there does not appear to be any serious root-rot that is universal in distribution, though several have been reported in various localities.

Howells (150) has investigated a serious one of tomatoes caused by a species of *Phytopthora* which he considers to be different from *P. parasitica* and *P. cryptogea. P. parasitica* has been reported as attacking fruits and stems of tomato as well as seedlings and roots (251, 259); *P. cryptogea* as the cause of a foot-rot of tomato (233).

Pink root on onions caused by *Phoma terrestris* Hansen has been reported by several workers (64, 122, 123). Recently Kreutzer (177) has enlarged our knowledge on this disease by pointing out that, in addition to onions, *P. terrestris* may also attack the roots of young plants of soya bean, pea, cane, millet, oats, barley, wheat, corn, squash, cucumber, cantaloupe, muskmelon, tomato, pepper, eggplant, cauliflower, carrot and spinach. In 1940 a pink root disease appeared on tomatoes apparently due to *P. terrestris* (303).

In 1939 a watery root-rot of cucurbits in Arizona caused by *Pythium aphanidermatum* (Edson) Fitz. also affected honeydew melons, quil muskmelons and crookneck squash, and caused rapid decay in apples, cantaloupes, carrots, cucumbers, eggplants, grapes, summer squash, sweet potatoes and tomato fruits artificially inoculated and maintained in a moist chamber (111). This same species is considered the cause of a root-rot of Coco-yams on the Gold Coast (341).

Recently a root-rot of pepper and pumpkin, caused by *Phytophthora capsici* Leonian, was shown to be also capable of infecting squash, eggplant and tomato (311). This fungus has been reported also as the cause of root-rot of chillies in Italy (63), of collar-rot of chillies in Macedonia (272), of blighting of aerial parts as well as girdling of pepper plants at soil level in Florida (333), of damping-off of pepper seedlings (176), and of mildew or blight of chilli peppers in the Argentine (110). Trotter in Italy reported a wilting of pepper due to root infection by *P. omnivora* de Bary, and Kreutzer (177) found that *P. terrestris* attacked the roots of seedlings of 19 different crop plants. *P. megasperma* has been reported as the cause of a root-rot of cauliflower in California in waterlogged soils, and artificial infections were obtained only when the soils were waterlogged (308, 309).

A foot-rot of rhubarb caused by *P. parasitica* var. *rhei* (108) and a tuber-rot of potatoes in Florida caused by *Xylaria apiculata* Cooke (265) have also been reported.

Herbaceous ornamentals. Root-rots have been observed on many ornamentals. Berkeley (19) in Ontario found that chrysanthemums, gladioli, roses and tulips were subject to a rot complex similar to that affecting raspberries and strawberries. He states: "It has become increasingly apparent that these root-rots are the result of a complex of factors in which parasitic organisms play a major rôle, and that this complex may vary with changing conditions of environment. For instance, not only the severity, but the type of organisms may change during the season. Greenhouse crops affected with root-rot often apparently outgrow this condition, and microscopic examination of the roots of such plants throughout the season has indicated a changing fungus flora in the roots".

Rhizoctonia solani, associated with three species of *Pythium,* has been reported on viola and pansy in two forms of root-rot, an epidemic form caused by *R. solani* alone, and a slow form with which are associated two or more of the fungi. It is suggested that the less severe is the result of mild antagonism between the organisms (47).

Buisman has reported on *Pythium* spp. causing a root-rot of cuttings. She found *P. intermedium* de Bary and *P. splendens* Braun. strongly parasitic on roots of geranium cuttings, while *P. debaryanum* and *P. splendens* were parasitic on chrysanthemum cuttings. In 1939 a root-rot and wilt of the Persian buttercup

(*Ranunculus asiaticus*) was caused by *P. debaryanum*, and it was demonstrated, by artificial inoculations, that, though not associated with the disease in the field, *P. irregulare* and *P. ultimum* Trow. were both capable of producing symptoms similar to those produced by *P. debaryanum* (306).

A root-rot, shoot-rot and shanking of forced tulips has been attributed to *Phytophthora cryptogea* Pethybr. & Laff. and *P. erythroseptica* Pethybr., either singly or in conjunction (39), and *P. cryptogea* is the cause of a foot-rot and root-rot of China aster, annual stock and Transvaal daisy (310), while *P. Richardiae* is responsible for a root-rot of Calla lilies in Holland (40), England (267) and the United States (304). A root-rot of paeonies (36) is caused by *P. peoniae* Cooper & Porter, and other fungi.

Tompkins, Tucker and Gardner (309), while working with a root-rot of cauliflower in California caused by *Phytophthora megasperma*, demonstrated experimentally that stocks, wallflower and cineraria were susceptible to the same fungus.

TEMPERATURE IN RELATION TO ROOT-ROT

That temperature plays a most important and in many cases a limiting rôle in relation to root-rots is readily seen by reference to the literature. *Pythium* rot of sugar cane was most severe at 65–68° F., and decreased in severity as the temperature was raised (247); in another instance it was more severe at 15° C. than at 30–35° C. and in wet than in dry soil (91).

It has been indicated that *Phymatotrichum omnivorum* (Shear) Duggar is readily checked by low temperatures and low moisture conditions (261, 294). High temperatures (around 80° F.) during the summer months favoured severe infection, but a drop in early November to about 50° F. produced sharp decline in infection in spite of abundant rainfall (294). The optimum temperature appears to be approximately 27° C., and temperatures of 39° C. or above killed the fungus (261).

Though occasional infection with *Aphanomyces euteiches* may take place through practically the entire range of temperatures at which peas will grow, infection is not abundant nor does invasion proceed rapidly below 15° C., the optimum being between 15° and 30° C. (164). Infection does not take place until the soil temperature is 14° C. or higher for a period of several days (120). The less important pathogen in the pea root-rot complex, *Fusarium martii* var. *pisi,* has a temperature range of 24° to 33° C. (162). In Wisconsin the important temperature range for several of the fungi associated with root-rot of peas were as follows: *Aphanomyces,* 15° to 30° C.; *Pythium* spp., 16° C. or above; *Rhizoctonia,* 9° to 18° C., and *Ascochyta,* 18° to 21° C. These figures clearly indicate, as Jones has pointed out, that root-rot may be initiated early in the season by *Aphanomyces* or *Rhizoctonia* when the soil is cool; then, when the soil warms up, *Fusarium* species may complete the attack started by low temperature fungi. No infection with *Aphanomyces* root-rot of peas was obtained at 12° C. (287), though slight infection was previously secured at 10° C. (164). Harter has reported that *Fusarium coeruleum,* which causes a root-rot of peas, has a temperature range of parasitism between 10° and 40° C. that corresponds fairly closely with the temperature range at which the host will grow.

Temperatures below 15° C. are unfavourable for *Pythium debaryanum* on sugar beets (38), and *R. solani* on the same host was most active between 30° and 35° C. (191). At 15° to 17° C. rate of decay was exceedingly slow.

Bliss, in a study of the relationship of temperature to *A. mellea,* found that severe infection of *Citrus sinensis* and *C. aurantium* (sweet and sour orange) occurred at 10° C., while infection was moderate at 17° C. and slight at 24° C. In *Prunus persica* (Lovell peach), *P. armeniaca* (Royal apricot) and rooted cuttings of *Pelargonium hortatum* (geranium), it was moderate at 10° and severe at 17° and 24° C. At 31° C. the inoculum was not viable and the roots were all healthy.

Johann, Holbert and Dickson (154) report that the "Pythium injury of dent corn depends upon environmental conditions. Germination may be prevented at low temperatures (12–16° C.) particularly in wet soils, by the rapid rotting of the embryo". At 12° C. they obtained a 57.1% loss of plants due to *Pythium arrhenomanes,* while at 16°, 20° and 24°, the loss was only 33.2, 2.1 and 2.2%, respectively, thus clearly indicating that infection by *P. arrhenomanes* is favoured by temperatures around 12° to 16° C.

In connection with root-rots of strawberry Nolan has reported that most damage by a species of *Diplodia* occurs when the temperature is above 80° F., while Hildebrand (137) found that the black lesion root-rot, as it occurs in Ontario, was favoured by temperatures above 48° F. Moreover, he found that strawberry plants escaped infection at 44° to 48° F. when grown in root-rot soil adjusted to contain 60% of its maximum water-holding content.

That temperature plays an important rôle in connection with black root-rot of tobacco is well established, since in a heavily infected soil little disease is found if soil temperature is below 15° C. or above 26° C. The most favourable temperature range is between 17° and 23° C. (158), though Doran (72) has pointed out that soil reaction influences the effects of temperature. In connection with brown root-rot of tobacco, Johnson (157) writes that the causal agent is destroyed in 24 hours at 45° C.

Though it is apparent that temperature has a direct effect on root-rots caused by strong parasitic fungi, and that such effects are primarily on the fungi rather than on the host, its rôle in complex rots of the strawberry type is not so apparent, largely because several fungi are involved. In such cases a temperature that favours one group may adversely affect other groups, and, therefore, temperature may be responsible in part at least for the various sequences encountered in such rots. Moreover, in this type the indirect effect of temperature on the general soil flora may play an important rôle in favouring one group at the expense of another, especially if the temperature is such that toxin producers are favoured and certain antagonistic capabilities are enhanced.

REACTION OF THE SOIL

The influence of soil reaction in connection with certain root-rots is well established. For instance, *Thielaviopsis* of tobacco and *Phymatotrichum* of cotton are reduced by increasing soil acidity (70–73, 221). Doran's studies on the effect of temperature and reaction on black root-rot show clearly the influence of soil reaction on infection at different temperatures. For instance, he found when the reaction was pH 5.6 or lower, there was no rot or only a trace at any temperature. Marked injury began at pH 5.7 at 15° C., at pH 5.7 or 5.8 at 18° C., at pH 5.8 at 21° and 24° C., and at pH 5.8 or 5.9 at 27° C. There was little or no injury at 30° C., even in soil with pH values of 6.0 to 6.9. Anderson also found that black root-rot was severe in soils with a pH value of 5.9 or higher, but was unimportant in soils with pH 5.6 or lower (6–8).

He concluded that no injury occurs below pH 5.6 but that between pH 5.6 and 6.0 there is a doubtful zone within which the rot may or may not occur (7). The critical point appears to be at pH 5.9. Others have shown that though *Phymatotrichum* grows best in culture at about pH 7.0, nevertheless, the root-rot of cotton for which it is responsible is found in soils of pH 5.5 to 6.4 as well as in neutral soils of pH 6.5 to 7.4 and alkaline soils of pH 7.5 to 9.0 (295). However, the percentage of diseased fields was twice as high and rot was much more destructive where the soil was alkaline or neutral, than where it was acid. On the other hand, they found *Fusarium* wilt much more common on the acid soils. Little or no growth of *P. omnivorum* was reported in a synthetic nutrient solution when the source of nitrogen was $(NH_4)_2SO_4$ (227, 83). Use of ammonium compounds for treatment of perennials infected with *Phymatotrichum* root-rot has been recommended, since $(NH_4)_2SO_4$ was toxic to the fungus (292), but others have shown that $(NH_4)_2SO_4$ *per se* is not toxic if the critical acidity is avoided (25). The latter suggest that the toxic factor was the acidity which developed as the ammonium sulphate broke down in the soil.

Root-rots of corn and sugar cane appear to be correlated with high absorption of aluminum (143, 27), and there is evidence that this correlation is dependent upon pH of the soil (209). Aluminum salts were not present in soil solutions where the reaction was higher than pH 5.8, and cases are cited where root-rot was present in soil with pH 4.6 but not when the pH was 6.0. Hoffer has noted the same situation in an Indiana corn field when root-rot was present in soil with pH 5.6 but not with pH 6.2. At the same time McGeorge points out that the relationship of aluminum solubility to root-rot is not the whole answer, since rot is present in many fields which have a pH greater than 5.8 and presumably where aluminum toxicity is not a factor. In this connection it is interesting to recall the case in North Carolina where potash added to soils with pH 3.5 to 5 tended to overcome root-rot, even though the level of aluminum in the soil had not been altered (277). Others have attributed the correction to the fact that potash has the property of greatly decreasing or eliminating the nodal accumulation of iron or aluminum in the plant, thus having the same effect as raising the soil reaction to pH 5.8, at which point aluminum salts become insoluble in soil solution (142, 144). *Pythium* sp.

responsible for root-rot of sugar cane in Louisiana was capable of growing well from pH 5.6 to 9.2, and most of the cane soils were in the neighbourhood of the neutral mark (91). Buchholtz reports that a pH below 6.5 favours root-rot of sugar beets (38), and Zutavern noted heavy damage to sugar beets by *Phoma, etc.,* in sandy clay soils at pH 6.3 to 6.6, while the roots were free from rot in clay sand at pH 7.0 to 7.3. He suggests that control consists in adjusting the soil reaction close to neutrality. In Germany (118) and Belgium (201) also, though black rot is prevalent in acid soils, it is rarely present when soil has a reaction of pH 7 or greater. On the other hand, the heaviest damage from *Phoma* root-rot occurred recently in very alkaline and neutral clay soils (117), whereas the epidemic of 1926 took place under acid soil conditions.

Reinking in New York found pea root-rot destructive in a wide range of soils with reactions ranging from pH 6.25 to 7.48 and in a few soils with a pH of 5.4 and 5.5 (252*a*). Burkholder, on the other hand, has shown that soil reaction has little effect either on the bean plant itself or on its susceptibility to *Fusarium martii* var. *phaseoli.*

Like temperature, the reaction of the soil has a direct effect, especially on the parasites associated with certain root-rots. Moreover, as Doran has shown, the pH of the soil may also directly influence the temperature range at which infection takes place. In addition, soil reaction may play an indirect rôle in its relationship to the accumulation of certain toxic substances that may be absorbed by the plant, thus rendering it more susceptible to attack. Such a correlation has been demonstrated between absorption of aluminum and root-rot of corn and sugar cane.

WATER CONTENT OF SOILS

Water content of the soil is one of the more important factors affecting occurrence and severity of most root-rots. In connection with *Fusarium* rot of pineapple, for instance, one species has been shown capable of invading the tips of roots and killing them when in wet soil, but not when the soil was dry (279). Others report that red stele root-rot of strawberry is more severe in heavy or poorly drained soils, though it may spread from such sections of a field to sections that are lighter or better drained (*e.g.,* 151, 134, 68, 5). In this connection Howells found the disease was evident first in the lower portions of a field and was commonly associated with a choked drain (151). Hickman writes: "thus, the disease (red stele of strawberries) was worst where soil drainage was impeded. Moreover, as time went on, the symptoms in the diseased plants became more intensified in these poorly drained areas than elsewhere". Excess moisture also favours the black root-rot of strawberry, since it has often been observed to make its first appearance in, and spread from low areas in a plantation. Moreover, strawberries growing at 44°–48° F. in root-rot soil, adjusted to contain 60% of its water-holding capacity, were shown to have clean, healthy root systems (137). However, by gradually increasing both temperature and moisture, the disease became more severe until at 60° F. healthy plants, set in root-rot soil containing 80% of its water-holding capacity, died within a week. At a temperature favourable for the rot, the disease was always more severe in soils containing 80% as compared with 60% of their water-holding capacity. Even at most favourable temperatures for strawberries, plants set in wet soil received a serious set-back.

In connection with root-rot of grapes, Rhoades found *Clitocybe* rot in the low, poorly drained parts of vineyards and orchards where water is liable to stand on the soil, although it may occur in soils underlaid with an impervious sub-soil or hard pan, where natural drainage is poor (253). *Rosellinia* and *Armillaria* rots of the vine produce rapid death of stock in heavy wet soils, while in lighter dry soils the lethal effects may be delayed for several years (181). In Galicia and in all humid and semi-humid regions of Spain *Armillaria mellea* is a serious pathogen of both vine and tree fruits (271).

Pythium aphanidermatum has been found to be severe on watermelons, honeydew melons, quil muskmelons and crookneck squash in rather poorly drained soils, where soil remained very wet for long periods (111), and this same relationship of soil moisture to root-rot is true of peas (67). In 1923 Jones pointed out that "wet soils promoted decay started by parasites (*Fusarium martii* var. *pisi*), though it does not appear to affect in great measure the action of the parasite itself" (162). Again Haenseler found that though root-rot of peas caused by *Rhizoctonia solani* was present in soils having from 5% to 80% of their water-holding capacity, the least injury was produced at 40% (119). There is also evidence that *Aphanomyces* root-rot of peas develops most aggressively in low, poorly drained soils and in heavier soils which are high in water-holding capacity (331), as well as in soil which is naturally retentive of water or in which water is held by reason of impervious layers (161). Moreover, in heavier soils the parasite may persist for at least six years after it has become abundant, while in light soils its period of survival may be shorter. In a survey of root-rot of peas in 1924 we read that "in approximately one half of the fields inspected the disease due to *Aphanomyces euteiches* was found in more moderate quantity, frequently being present in severe form only in situations unduly wet as a result of inadequate drainage or proximity to water courses" (74). More recently *Aphanomyces* root-rot of peas was revealed as being favoured by high soil moisture, since no rot occurred in naturally infected soil when the moisture was maintained at 45% of its water-holding capacity, while at 75%, 72% of the plants became infected (287).

Low soil productivity and soils either too dry or too wet seem to favour late root-rot of sugar beets with which *Phoma betae* and *Fusarium* spp. are associated (288). In this connection *Phoma* rot was benefited by drought "which especially during June appears to create a dangerous period in the life of the beet, since an early drought reduces the vitality of the beets so much that they fail to recover and later succumb to late blight and root rot" (256). Le Clerg reports that *Rhizoctonia* root-rot of sugar beet is favoured by low soil moisture (190), and Young, using soil temperature tanks, found the lowest incidence of black root and the best beets developed at 54° and 61° F. in the slightly dry and slightly wet soils (342). Others noted that drainage was beneficial in the reduction of sugar beet damping-off or black root (*Pythium* spp., *Rhizoctonia* spp. and *Aphanomyces* spp.) and that good results were obtained by growing the plants on ridges with furrows on both sides (57).

Root-rot of sugar cane, too, is favoured by poor drainage conditions (77), and in Cuba such predisposing factors as lack of drainage, lack of moisture, deficient cultivation and deficient fertility are all associated with the general complex (84). A high water table combined with fine texture of soil and low winter and spring temperatures are probably the chief conditioning factors of severe outbreaks on present commercial varieties of sugar cane in Louisiana (247). Flor also points out that root-rot of sugar cane caused by *Pythium* sp. is more severe in soils at 50% or more of their water-holding capacity (91), and it has been favoured by abnormally high early winter temperature followed by alternate wet and dry periods (245). Ciferri, in a publication on mycorrhiza in relation to root-rot of sugar cane, points out that any factor which hinders rapid formation of new roots, favours an outbreak of rot, and he mentions in this connection excessive moisture, severe drought and other factors.

Excessive moisture, high temperature and poor drainage have been found to favour root-rot of peppers caused by *Phytophthora capsici* (311). *Phytophthora* root-rot of sweet clover was most abundant in low, wet portions of fields (163), and low, poorly drained areas favoured root-rot of cauliflower caused by *P. megasperma* (308). Petri notes that excessive soil moisture should be avoided by drainage in combating *Phytophthora* root-rot of citrus (236).

The violet root-rot of tea, due to *Sphaerostilbe repens* B. & Br., is stated to be associated with water-logged and acid soils (318), while the same fungus causes a red rot of limes in St. Lucia only in poorly drained orchards (332). In the Sudan a root-rot or wilt caused by a fungus complex was associated with years of high rainfall and soils of alkaline content (203), and elsewhere a root-rot of chestnut and beech was associated with impeded drainage in the sub-soil (65).

It thus becomes clear that root-rots in general are aggravated by excess soil moisture, though it should be realized that factors other than lack of aeration are connected therewith, since toxins, including hydrogen sulphide, soluble aluminum, iron and manganese compounds and organic substances, may occur in poorly aerated soils. Experimental evidence indicates that aeration of the soil produces no obvious difference in the amount of root-rotting, thus suggesting the presence of some factor or factors in addition to excess water. Others have shown that salicylic aldehyde predisposed sugar cane roots to infection by *Pythium arrhenomanes,* with reduction in weight from two to seven times as great as that caused by the fungus alone (248).

In the above résumé of the literature dealing with the correlation of soil moisture to root-rots, it is apparent that practically all rots are favoured by excess soil moisture. This is readily understood when it is recalled that most soil fungi are favoured by abundant soil moisture, whereas the roots of plants are adversely affected by the same condition, since roots require good aeration to function normally. Hence excess water renders the host more susceptible and favours the growth of the soil flora in general. Moreover, in water-logged or poorly drained soils toxins accumulate which apparently increase the susceptibility of the host and encourage root-rot attack.

CONTROL

Root-rots, largely because of the complex nature of their environment, are most difficult to control, except in those few cases where resistant varieties are available. Still much can be done to lessen the losses caused by these diseases. The most general means of control consists of planting healthy stock, supplemented by crop rotation, certain cultural practices, fertilization and cover crops. Where the rots are seed-borne, such as those of peas and beets caused by *Ascochyta* and *Phoma,* respectively, seed disinfection is of value. In addition, there are many miscellaneous measures of value in connection with certain specific rots which will be mentioned.

Varietal resistance. Obviously the ideal means of controlling root-rots is by use of resistant varieties, but, though some progress has been made along this line, especially in connection with brown root-rot and black root-rot of tobacco, there are as yet few rots where this means of control is available, due to the lack of suitable resistant varieties. Peglion in Italy in 1897 was the first to appreciate the possibility of controlling black root-rot by means of resistant varieties. Since then, breeding and selecting for resistance has been under way, especially in Italy, the United States and Canada. In 1916 James Johnson studied resistance to black root-rot in white burley and cigar-leaf tobaccos and found that Little Dutch and Connecticut Broadleaf were most resistant. In 1930 he published on the genetics involved in resistance to the disease. In the United States to-day breeding and selection for resistance to root-rot is being carried out, especially in Wisconsin, Kentucky, Virginia, Connecticut, Maryland, Tennessee and North Carolina. In Canada, it centers mainly in Ontario at Ottawa and Harrow. Some of the resistant varieties listed in the United States are Havana 142, Round Tip, Maryland Standup Resistant, Johnson resistant White

Burley, Kentucky No. 5, Kentucky 16, Standup Burley Mammoth, Yellow Special and Special 400. These varieties or strains are being used in connection with present breeding programmes (98). In Canada the list of varieties in order of degree of resistance to black-root is (21): Gold Dollar 82C, Yellow Mammoth, Duquesne, Bonanza, White Stem Orinoco and White Mammoth of the flue-cured varieties, and Harrow Velvet (developed at Harrow Station), Kentucky No. 16, Halley's Special, Type No. 5, Gay's Yellow, Kelley and Judy's Pride of the burley type of tobacco. Of the cigar binder types, Resistant Havana, Havana 211, Havana 236, Connecticut Broadleaf (William), Comstock Spanish Pomeroy and Connecticut Havana No. 38, and of the pipe varieties, Grand Rouge, Belge, Rose Canelle, Parfum d'Italie, Obourg de Vincent, Petite Havana and Canelle, are listed. In connection with brown root-rot, the burley varieties Kelley and Judy's Pride are more resistant than Harrow Velvet, Halley's Special, Kentucky White Burley, Gay's Yellow, *etc.*, while of the flue-cured varieties White Mammoth, Bonanza, White Stem Orinoco and Duquesne appear to be more resistant than Yellow Mammoth, White Stem Willowleaf, Adcock, Jamaica Wrapper, *etc.* (175).

In this connection it is interesting to note that certain varieties resistant to black root-rot may be particularly susceptible to brown root-rot, and *vice versa*. For example, the Harrow Velvet variety, outstandingly resistant to black root-rot, appears to be one of the most susceptible varieties to brown root-rot. Valleau in 1925 was possibly the first to present evidence to the effect that a strain of tobacco resistant to black rot might be susceptible to the brown (320).

Recently Reid, reporting on the general research carried out since 1929 by the Department of Agriculture for Scotland on the red stele or red core root-rot of strawberries, states that five seedling varieties (Auchincruive 1, 2, 4, 5 and 6) have shown striking resistance to this rot in England, Illinois and Maryland. The American variety Aberdeen is also resistant in Scotland and America. In breeding for resistance to this disease in America, many promising seedlings were obtained and four hybrids selected, all crosses between Aberdeen and Fairfax, that are immune and commercially promising (153). Other hybrids of interest in this connection were a cross between a Scottish selection BK–46 and Fairfax; Aberdeen × Mastodon selection; Aberdeen × Blakemore selection and Aberdeen × Dorsett.

In research over a period of years at St. Catharines, Ont., it was demonstrated that the native wild variety *Fragaria virginiana* was much more resistant to black root-rot of strawberry than were the commonly grown horticultural varieties. Of these, the Premier variety appears the most susceptible, while Blakemore shows slight resistance (137).

Increased resistance to root-rot of sweet clover by means of selection has been reported (163), and in recent other studies relating to varietal resistance of alfalfa and sweet clover to root- and crown-rots, *Medicago falcata* showed the highest resistance to *Cylindrocarpon Ehreubergi* Wr. and *Sclerotinia sativa* Drayton & Groves, while sweet clover varieties belonging to *M. officinalis* showed more resistance than those of *M. alba* to *S. sativa* (60). A strain of Alpha sweet clover, developed at Saskatoon, was quite resistant to *Phytophthora cactorum.*

Considerable work has been done in connection with breeding and selection for disease-resistant sugar cane, and the following commercial varieties have been cited as being resistant to root-rot: C. O. 290, C. P. 807, C. P. 28/11 and C. P. 29/116 (247).

J. L. Weimer found hairy, smooth, light-seeded Hungarian vetches and *Vicia hybrida* to show considerable resistance to root-rot (*Aphanomyces, Pythium, Rhizoctonia, Fusarium* spp., *etc.*), whereas woolly pod, Monantha and common vetches (*V. sativa*) were quite susceptible. He reports that no variety of pea is resistant, a fact which has been confirmed by many others. On the other hand, there are many varieties of peas which show resistance to wilt caused by *Fusarium orthoceras* var. *pisi.* In this connection 12 varieties are listed as being resistant under Wisconsin conditions (331).

It is interesting to note that though some 1700 species of plants are susceptible to the cotton root-rot fungus, monocotyledonous plants, *e.g.,* corn, onions, cannas and certain grasses, are highly resistant or immune. This resistance is explained as being due to the presence in such plants of an ether-soluble toxin or toxins, possibly organic acids or esters (82). In this connection it has been demonstrated that resistance of some species is associated with the presence in those plants of certain alkaloids (113, 115), and that phenolic acid and related compounds are toxic to *Phymatotrichum omnivorum* (114). Certain plants, especially in Ranunculaceae, Berberidaceae, Fumariaceae, Leguminosae and Lolanaceae, have shown a correlation between the presence of alkaloids in them and their resistance rating to *P. omnivorum* (112). Though no variety of cotton has proved to be consistently resistant to root-rot, several legumes, such as sesbania and guar and some varieties of cowpeas, appear to be highly resistant.

Though some have found no evidence of resistance in *Malus* spp. to *Xylaria mali* (93), another investigator noted that suppressed or weakened trees were more susceptible to attack by this fungus (33). Barss reports French pear root stocks (*Pyrus communis*) and the northern California black walnut stocks to be highly resistant to *Armillaria mellea,* while the common English walnut is very susceptible. Kesseler says that Wild Brazil, Pernambuco, Congo and Ruby pineapple varieties show resistance to pineapple root-rot (*Nematosporangium* (*Pythium*) *rhizophthoron*), while the Cayenne variety is susceptible. In three hybrid varieties, the parents of which were also tested, he found that susceptibility was intermediate between that of the parent forms.

Crop rotation. As Garrett (104) states, "crop rotation is one of the oldest methods of controlling soil-borne diseases and is dependent upon specialization of parasitism in the pathogenic organisms. Its value becomes restricted with increase in host range of the parasite, so that the control of such a fungus as *P. omnivorum* with a known host range of 1700 species by crop rotation severely curtails the range of possible crops which can be safely grown". It is obvious also that a much longer period of rotation will be required when the organism to be controlled produces thick resting spores or sclerotia, than when such forms are not involved. Indeed, large numbers of viable sclerotia of *P. omnivorum* have been found in soil after 12 years of continuous cropping with non-susceptible cereals, and after the same period of clean fallow (262).

Moreover, certain fungi associated with root-rots are capable of existing as saprophytes on organic matter in the soil, thus necessitating a much longer period of rotation, if not actually eliminating this method, in some cases at least, as a satisfactory measure of practical control. In this connection, *Fusarium culmorum* (W. G. Sm.) Sacc., a well known parasite of cereals and other crops, was the dominant fungus associated with early decomposition of buried wheat straw, thus indicating that it has a saprophytic as well as a parasitic stage and hence is not apt to respond readily to crop rotation (266). It is sometimes impossible to grow flax susceptible to wilt (*Fusarium lini* Bolley) more than once in 10–12 years (13), and survival periods of 11 and 14 years have been found for *Fusarium conglutinans* Wollenw., the cabbage yellows organism (215, 166). In the tropics, species of *Rosellinia* are capable not only of growing as saprophytes through dead leaves and other decaying vegetable matter in or on the surface of the soil, especially in shady or damp situations, but of parasitizing tea and other plantation crops with which they come in contact in the course of their growth through the surface litter (94, 318). *Pythium arrhenomanes,* too, remains active in the soil at least four years and has not been controlled by ordinary two to three-year rotations or fallow (80). Notwithstanding the above and other adverse factors, crop rotation has proven itself an essential practice in the control of root-rots. For instance, *Pythium* rot of sugar cane is generally of little importance throughout the sirup-producing sections of Alabama, Florida, Georgia and Mississippi, apparently because of the long rotation (3 to 5 years) of crops (non-susceptible) commonly followed, and in southern Louisiana the common practice of a one-year rotation of sugar cane with a legume is not long enough to prevent root-rots being of major importance (247). *Aphanomyces* root-rot of peas can be prevented and controlled most effectively by crop rotation. The length of rotation varies, however, with local conditions. For instance, on certain irrigated soils with low moisture-holding capacity, root-rot is lacking or has not become destructive even after peas have been grown nearly every year for ten years. On the other hand, the disease has become destructive on similar soils when they were sub-irrigated. Moreover, on some soils in humid territory the third successive crop of peas is often badly damaged by this rot, and a comparatively long rotation appears to be necessary to prevent it from accumulating in the soil. In this connection it is to be noted that a six year rotation has been insufficient for soils that were heavily infected (164).

Leach reports that a two to four-year rotation of sugar beets with cereals, or winter crops of peas, spinach or lettuce reduced

Sclerotium rolfsii to safe levels, but this was not the case when sugar beets were rotated with summer crops of beans, on account of both susceptibility to pathogens and their cultivation during the summer when higher temperature favoured the fungus concerned (183). Similarly, flax following sugar beets was subject to attack by *Pythium* spp., since both crops are susceptible to these fungi (273). In connection with the *Phoma* root-rot of sugar beets, the disease was most prevalent where crop rotation was neglected, and the best crop preceding sugar beets was peas (117). Campbell states that black root of sugar beets (*Rhizoctonia* sp., *Pythium* sp. and *Aphanomyces* sp.) can be controlled by proper rotation. He recommends that a cultivated crop such as beans, corn or potatoes intervene between a sod and a beet crop. Root-rot of sugar beets caused by *R. solani* was not present in beet fields preceded by four years of grain (207), while sugar beets following potatoes developed less rot than when sugar beets followed sugar beets (192).

It is well recognized that black root-rot of strawberry is more severe when strawberries follow strawberries (137); hence the recommendation to rotate strawberries with some other crop. It is further recommended that when practical, strawberries be planted on land that has never grown them, since it has been the experience of all investigators that root-rot is rarely present the first or second year on such soil.

In Java, it is recommended, at least three years should elapse between crops of sugar cane on the same soil (179). Investigations on root-rot of flax caused by *Thielaviopsis basicola* recommend two successive root crops previous to the flax (234). Corn in Missouri should not be grown more than two years in five on the same soil (30), and infection by *Pythium* root-rot appears reduced when soya beans precede corn in the rotation, but increased when the corn is preceded by timothy (258).

In connection with *Fusarium* root-rot of clover, Young suggests temporary substitution of a non-susceptible leguminous crop such as soya beans. Others recommend rotation and drainage of soil as essential in preventing root-rot of peas caused by *Aphanomyces euteiches*, *Pythium* spp., *Fusarium* spp., and *Rhizoctonia* spp. (74, 290, 196, 164). However, a three-, four- or five-year rotation did not entirely eliminate pea root-rot, though the population of the pathogens in the soils was reduced (252*a*). Pugsley, in advising on prevention of root-rot of onions caused by *Fusarium* spp., also recommends crop rotation and sanitation.

In connection with root-rot of cotton, a two-year fallow with a rotation of non-susceptible crops (212) or tillage operations that will reach the sclerotia as a supplement to fallow and rotation (213), is advised. A two- or three-year rotation with grains combined with deep tillage may also be effective (171), and the general principle of fallow and crop rotation is advocated by numerous other investigators (260, 292, 250, 298, 167, 62).

In a testing of the influence of the cropping system on tobacco, brown root-rot was prevalent following timothy, clover, corn and hay and only slightly less following potatoes (165, 159). In Ontario, when timothy, corn or soya beans preceded tobacco, brown root-rot was more prevalent and severe (175). In the end decomposition of corn and timothy infusions some toxic agency or agencies appear to be liberated which tend to induce brown root-rot, and other crops which do not induce the rot under field conditions likewise do not do so during the various stages of decomposition under laboratory conditions (71, 78, 79). Also, brown root-rot appears to be less severe in tobacco after a crop of lucerne or alsike clover than after timothy (71).

Biological control. The present status of toxins in relation to root-rots has recently been reviewed (107), and the same subject discussed in connection with the general aspect of biological control (104). The principle of biological control is based on the fact that the soil population is in a dynamic biological equilibrium. Therefore, factors which favour the build-up of saprophytic organisms are likely to upset the balance to the disadvantage of the parasitic forms and to result in a reduction of disease. This end may be accomplished in various ways, as by green manuring, manuring, changing pH, clean fallow and use of chemicals. Green manuring or addition of sufficient organic matter to the soil has been demonstrated by many investigators to have a beneficial effect on certain crops affected by *Actinomyces scabies* (Thaxt.) Güss. (218, 219), *Ophiobolus graminis* Sacc. (90, 100–102), *Phymatotrichum omnivorum* (172) and black root-rot of strawberries in which several organisms are associated (139).

Canadian workers (especially 270, 33, 34, 132) and others (305, 75, 76, 173, 335, 336) have presented evidence that certain organisms are antagonistic to specific parasites and capable, under suitable conditions, of effecting reduction of disease. Clean fallow was beneficial in connection with cotton root-rot (211) and with *Helminthosporium sativum* and *Ophiobolus graminis* (269), and, though the method whereby this reduction was effected is not clearly understood, it would appear likely (107) that certain antagonistic effects—conditions unfavourable for the parasite or lack of food for the parasite under summer fallow conditions—may be factors of importance.

The effectiveness of organic manure in checking root-rots is generally explained by the hypothesis that addition of large quantities of green plant material increases the numbers and activity of saprophytic forms at the expense of parasitic forms which supply, in part, the nitrogen required for the decomposition process. In addition, a qualitative as well as quantitative effect on the soil microflora may be involved when green manures are turned under as shown by a preponderance of fungi in the roots of tomato, soya beans and corn. The predominating fungi were *R. solani*, *Thielaviopsis basicola* and *Pythium* spp., respectively (139). If the biology of the soil is in a dynamic equilibrium, then the buildup of specific fungi must effect the balance of other groups. That it is possible to affect the soil flora in this way is indicated by the fact that turning under soya beans reduced the incidence of root-rot of strawberries (139). However, the principles underlying this beneficial result have yet to be investigated.

Several investigators have presented evidence to the effect that ammoniacal nitrogen may be toxic to certain fungi, thus suggesting the possibility of using ammonia-yielding compounds for control of cotton root-rot (226, 227). Streets recommends ammonium sulphate (292), and increases of five to nine tons of sugar beets per acre by application of it have been secured (185). It was not determined, however, whether the beneficial results were due to toxicity of the ammonia or to the influence of the nitrogenous fertilizer upon the host. More recently, from 1934 to 1937 nitrogenous fertilizers gave a consistent reduction in rot of sugar beets caused by *Sclerotium rolfsii* (186). In explanation thereof it was suggested that either nitrogenous fertilizers may effect changes not only in the metabolism of the fungus, reducing its growth or pathogenicity, but also in the host itself, possibly increasing its resistance, or the fungus may be depressed as a result of a change in the balance of the soil flora. In this connection there was experimental evidence to the effect that low concentrations of ammonia are toxic to the mycelium and sclerotia of *S. rolfsii.* However, since ammonium sulphate in alkaline solution and calcium nitrate in solutions of similar concentrations were only mildly toxic and harmless, and since all three materials gave equally good results in field trials, the authors suggest that the control obtained in the field may have been due to factors other than ammonia toxicity. Likewise, Blank and Talley question that ammoniacal compounds *per se* are toxic, since they found that ammonia nitrogen is a good source of nitrogen for *P. omnivorum.* They suggest that the beneficial results from ammonia compounds may be due to a growth response to the additional nitrogen and/or the acid condition which results from application of such compounds (*cf.* section on Reaction of Soil, above).

More recently (1941) Henderson, in studying the value of certain nitrogenous compounds as disinfectants for tobacco seedbeds, found that *Thielaviopsis* root-rot of tobacco could be controlled effectively by application of urea to the soil at the rate of ½ lb. per square yard. However, when ¼ lb. urea was used the rot was severe, though at the end of the experiment the soil showed a reaction of pH 8.00 in comparison with pH 8.65 in the soil treated with ½ lb. of urea. This would suggest that the greater accumulation of ammonia in the soil where ½ lb. was applied, was responsible for the sterilizing effect, and not the slight difference in pH reaction. Additional evidence that this was the case was obtained with studies in the laboratory which showed that a concentration of ammonia as high as 93 mgms. ammonia-nitrogen per 100 grams of dry soil at a pH of 8.1 was sufficient to prevent growth of *T. basicola.* The above concentration of ammonia was found by analysis of seedbed soil to be present in the urea (½ lb.) treated soil. Thus Henderson's results in the soil treated with ½ lb. of urea support the contention that certain ammonia compounds may be toxic to certain fungi. Others suggest that the beneficial results of ammonia compounds may be explained on the basis that root diseases are toxin-induced and that the basic nitrogen in ammonia antidotes the toxin (149).

Fertilization. Sardina and Landaluce claim that *Armillaria* root-rot of the vine can be controlled only by preventive measures which, in addition to extirpation, deep cultivation and drainage of the holes, should include replacing of stable manure with well-balanced mineral fertilizers (271). In Louisiana nitrogenous fertilizers increased *Pythium* root-rot of sugar cane, while high phosphate treatment reduced it, and neither nitrate nor phosphate had any marked effect on the antibiotic action of *Trichoderma* spp. against *Pythium* (189). This same relationship of nitrate to *Pythium* root-rot was also reported from Coimbatore (243) and the United States (247).

On the other hand, marked control of *Aphanomyces* root-rot of peas was obtained under greenhouse conditions with one application of 4–16–4 fertilizer at the rate of 500 lbs. per acre (330). In field plots, where the fertilizer was applied with the seed, increases in yield from 46% to 248% were obtained over control plots. It appeared that quickly available nutrients in amounts of 200 to 300 lbs. per acre in the furrow with the seed made it possible for the plants to develop greater resistance. Much greater reduction of rot and increases in yield were secured when 2% of readily available nitrogen was included in the fertilizer than when it was omitted. More recently there is evidence that the nitrogen fraction is more active in reduction of pea root-rot than either the phosphorus or potash content when 2–12–6 fertilizer is used (287). In New Jersey root-rot of peas was reduced by heavy applications of hydrated lime (4000 lbs. per acre), but lesser amounts had no inhibitory effect (120), and elsewhere incidence of the rot was delayed and injurious effects on the host greatly reduced by use of 1000–2000 lbs. of complete fertilizer per acre (121). Greenhouse tests indicated that nitrate of soda, sulphate of ammonia and muriate of potash were more effective than was superphosphate. Reinking in New York states that in favourable growing seasons, profitable yields of peas were obtained where soils were sufficiently fertilized (600 lbs. per acre of a 5–20–5 or 10–20–10 fertilizer) in spite of the presence of pathogens in the soil (252*a*).

Reduction in root-rot of sugar beets has been reported with application of commercial fertilizer containing 100 lbs. nitrogen per acre (183), and promising results in control of black-root of sugar beet were secured by incorporation with the seed of liberal amounts of phosphate fertilizer—three to four times the customary dose of 100 or 150 lbs. per acre (57). More recently, experiments from

1934 to 1937 in the Sacramento Valley, California, showed that application of nitrogenous fertilizers gave a consistent reduction in *Sclerotium* root-rot of sugar beets (186).

It is considered that root-rot of Coco-yams may be due to lack of potash. Therefore, presumably, addition of potash would be of value as a corrective (341). Soil deficiencies, especially of potash, and accumulation of aluminum and iron compounds in corn appear to render the corn plants more susceptible to root-rot (143, 199).

In France an investigation of the composition of soils in which fruit trees and vines suffered from attacks of *Armillaria mellea* indicated that such soils were poor in carbonate of lime (97). Previously Gard (96) had expressed the opinion that a deficiency of carbonate of lime was the chief factor pre-disposing walnuts to attacks by *A. mellea.*

Use of commercial fertilizers in controlling root-rot of cotton has been studied by many investigators. Though there is evidence in some sections of response from using nitrogen and phosphoric acid in combination, the results in general have been inconsistent and not convincing. Incidence of the rot on light, sandy loam was reduced by nitrogenous fertilizers and increased by those containing phosphorus, while on heavier soils the effect was less pronounced (168). Ammonia salts, *e.g.*, sulphate and phosphate, were toxic to *Phymatotrichum omnivorum*, and the sulphate was definitely beneficial to certain infected trees and could be applied with success to small areas of infection in cotton and alfalfa fields (227, 292).

Disinfection of soil. Disinfection or sterilization of soil is a most satisfactory method of overcoming root-rots, but not practical except for small plots or seed and plant beds. Sterilization of seedbed soil by means of steam or chemicals, especially formalin and chloropicrin (50, 52, 109, 194, 210, 343), is common practice in many districts, particularly for black root-rot of tobacco and root-rot of sugar beets. In addition to use for disinfection of seedbeds, various chemicals have been recommended as auxiliaries. For instance, there is the removal of vines infected with *Rosellinia necatrix* or *Armillaria mellea* and disinfecting the soil under such vines with carbon disulphide or formalin (181). Thomas and Lawyer have also recommended carbon disulphide in connection with *A. mellea* (302). They report a kill of the fungus to a depth of 60″ under a wide range of conditions when 45 cc. of carbon disulphide is applied 8″–9″ deep at 18″ staggered intervals. They say that eradication of the fungus from orchard soils seems possible if applications are made under ideal conditions, that is, low soil moisture and a surface blanket of moistened soil. In connection with a root-rot of forced tulips, treatment of the soil previous to planting with steam or formalin yielded 99.5 and 97%, respectively, of marketable bloom, while Cheshunt compound gave unsatisfactory results (39). Howells (150), reporting on a *Phytophthora* root-rot of glasshouse tomatoes, recommends cutting away affected roots while plants are in pots, to about ½″ above the level of decay, repotting and watering with mercuric chloride solution (1 oz. per 20 gal.), nitrate of soda being applied two days later. Esmarch recommends for small areas removal and burning of affected plants, combined with soil disinfections by means of a 1% solution of formalin or Uspulum to control violet root-rot (*Rhizoctonia violacea* Auct. Amer.) of clover, lucerne, turnip and asparagus.

In black-rot of sugar beets satisfactory control of late pre-emergence and post-emergence rot was obtained by using cyanamide, 135 lbs. per acre, a week before planting (42). In addition, Campbell and many other investigators have demonstrated the value of seed treatment in connection with this disease. In so far as seed treatment for control of black-rot (blackleg) of sugar beets is concerned, there is conflicting evidence, though the bulk of evidence favours it. Many have reported on the value of seed treatment (241, 263, 42, 106, 232, 178, 289, 201, 340), though Campbell found no evidence of protection after the plants had emerged, and there is a report of beneficial results on the lighter type of soil with none accruing on certain heavier types (58). In this connection Stirrup has shown that when seedlings are growing under adverse conditions, seed treatment exercises a certain amount of control, but when conditions are favourable for growth, little or no benefit accrues from such treatment. Appel in 1929 also reported on unsatisfactory results from chemical treatment, but beet plots in California on which ammonium sulphate or ammonia were added to the irrigation water showed less than 1/3 of the *Sclerotium* (*rolfsii*) root-rot found in the controls, and cyanamide worked into the soil three weeks before planting, reduced the incidence of infection by some 50% (182). In Italy Petri recommends drainage and manurial dressings, counterbalanced by application of phosphate, for prevention of *Phytophthora* root-rot of citrus (236). He states a cure may be effected by laying bare affected roots, pruning, removing all dead and diseased roots, cutting away the necrosed parts of the collar and disinfecting the surface with a 25% to 30% solution of ferrous sulphate or a 2% to 4% solution of copper sulphate and lime. After drying, the wounds should be painted with pitch or carbolineum. In replacing a diseased tree he recommends removal of the old roots, the cavity to be left open for a year, and application of 2% Bordeaux mixture, 5% solution of formalin or quicklime.

Organic manures. In Arizona King (171, 172) recommended burying, during the winter, large quantities of organic matter in deep furrows in the affected areas. The ground is irrigated to encourage rotting, and cotton is planted in the spring over the decayed organic matter. Control increases with successive treatments, since this practice builds up an abundance of active saprophytic organisms, a condition which is not favourable to *Phymatotrichum omnivorum.*

Incidence of strawberry root-rot appears closely correlated with soil treatment, for plants grown in sterilized soil and soil in which soya beans had been incorporated, remained free from disease until the third season, while those in soil following manure, corn, red clover and timothy all became diseased, the severity of attack increasing in the respective series in the order named (139). In small outdoor plots at St. Catharines over a two-year period yield increases of 525 and 825 quarts per acre have been obtained when strawberries were grown in plots in which one or two cover crops of soya beans were turned under, respectively, in comparison with similar plots in which strawberries were ploughed under.

In explanation of this beneficial action, decomposing soya beans may undergo a carbohydrate breakdown which favours accumulation of innocuous fungi at the expense of pathogenic forms (337). This same beneficial effect was produced in root-rot soil by substitution of glucose for soya beans. When red clover was incorporated with the soil, a putrefactive decomposition took place with no beneficial shift from a root-rot standpoint in the biology of the soil.

Ringing, felling and barriers. In connection with control of *Armillaria* and *Rosellinia,* use of trenches has been recommended to delimit affected trees or areas (14). Open trenches have been used also for checking the spread of *Phymatotrichum* root-rot of cotton. In addition, artificial barriers, such as galvanized iron, or chemical barriers, such as crude oil, sulphur, sulphuric acid and copper sulphate, have given satisfactory results in checking this same disease. It is stated that the chemically treated soil barriers are successful and practical. The soil is removed to a depth of 30 inches and a width of 6 inches, mixed with the chemical and replaced. Barriers 4 to 6 inches wide, in which 2 to 4% of sulphur had been incorporated with the soil, prohibited the fungus from crossing the barrier. The treated soil constituting the barriers became as acid as pH 2.2, while outside the barrier it remained around pH 7.5–8. Four or more rows of sorghum acted as effective barriers (296).

Ring-barking affected trees as a measure of controlling root-rot has been recommended for *Armillaria* and kindred types. Leach, studying *Armillaria* root-rot of tea in Nyasaland, observed that the hyphae developed freely in the xylem and pith and not at all in the cortex of seedlings, suggesting that the fungus requires abundant supplies of carbohydrates (187). It was also found that the roots of normally felled trees were high in carbohydrate content, and it seemed feasible, therefore, that if trees were bark-ringed before felling, to prevent passage of carbohydrates to the root from the leaves, such roots would be less susceptible. An experimental test supported this hypothesis. Examination of 240 felled trees (unringed) showed that 54 were affected by a dry rot and 186 showed *Armillaria* rot. Of 18 trees ringed before felling there was only one doubtful case of *Armillaria* rot. More recently Leach expressed the opinion that the rate at which roots die after a tree is felled is the factor controlling distribution of *A. mellea* in cleared forests, and accordingly ring-barking of deciduous trees should be effected just after breaking into leaf (188). He recommends that trees which die slowly after ring-barking should be felled one year after ringing. Forbes, also working in Nyasaland, recommends that trees and shrubs on all land to be cleared for planting to tung trees, be ring-barked at least 18 months before planting operations, as a preventive against collar crack (*A. mellea*). Gadd in Ceylon also found the practice of ring-barking felled trees promising as a means of protecting the tea crop from infection of *Poria hypolateritia* in jungle clearings (95). Napper has found that stump injection with sodium arsenite is advantageous in control of *Fomes* root-rot of *Hevea* rubber, since it hastens decay and invasion by saprophytic fungi, thus reducing chances of infection by and spread of *Fomes lignosus* Klotzsch. in the replanted stand (223, 225). This is, in fact, the basis for girdling or felling as practised in connection with root-rots where inoculation comes from root infection in indigenous forests or plantings to be replanted. All these measures aid in rapid decomposition of roots and stumps, thus favouring decay organisms at the expense of parasitic fungi.

Miscellaneous measures. Another rather unusual means of controlling root-rots involved planting a mixture of three different indicator plants in areas to be replanted to *Hevea* rubber in Ceylon (23). The value of this method lies in the prompt spotting of indicator plants affected with rot and the removal and burning of all infected material.

In California infection by *Sclerotium rolfsii* was 2.4 to 4.2 times as great in unthinned sugar beet plots as in plots thinned to produce a uniform stand of single plants (184).

Certain tropical species of *Rosellinia* are capable of spreading through leaf mold (94) on the surface of the ground, and Gadd recommends in a wet season the removal of such litter, especially from around the main stems of tea bushes. He points out that in dry seasons there is little chance for the fungus to spread as noted above. Tunstall states that liming the soil and exposing the collars of tea bushes often checks the spread of *Rosellinia.*

Though rogueing is not practised generally in connection with root-rot control, it has given some success in relation to certain tropical root diseases of plantation crops in Malaya. Thus Napper has found rogueing of value in connection with control of root disease on rubber caused by *Fomes lignosus* and *F. noxius.* He also reports that tree to tree collar inspection of rubber trees, accompanied by digging around infected trees to expose and remove diseased roots, has resulted in low incidence of infection at a very low cost (224).

TYPES OF ROOT-ROTS

In the foregoing résumé it is clearly indicated that several different fungi may be involved in the production of root-rot on certain hosts. The question naturally arises as to whether it would be preferable to look upon such a rot as a single disease, though several fungi may be involved, or to take the view that each fungus of the group associated with the rotting should be considered as the cause of a distinct root-rot, even though there may be no macroscopic means of distinguishing between them. Both views are at present recorded in the literature. If the former be accepted—the weight of evidence is in its favour—then root-rots may be classified into three general groups: Type 1. Simple root-rot. This is the type where a specific organism is responsible for a rot, such as the *Xylaria, Armillaria* or *Clitocybe* rots of apple, and *Phymatotrichum* rot of cotton and other plants. Type 2. Compound root-rot. In this type, though there is a specific organism of prime importance, other fungi are commonly associated with it and aid in producing the rot as it occurs in nature. Examples of this type are the *Pythium* root-rots of sugar cane and corn, *Aphanomyces* rot of peas, and possibly *Thielaviopsis* rot of tobacco. Type 3. Complex root-rot. In this type no specific fungus is alone constantly associated with the diseased condition, but a complex of fungi and other organisms is involved, no one of which appears to stand out as being primarily important. In this category would be placed the black lesion type of root-rot of strawberry in England (20) and Ontario (135, 313) and raspberry root-rot in Ontario and British Columbia (18).

At this point it might be well to consider the time factor or sequence in which fungi become involved in certain complex root-rots. There is evidence to the effect that *Pythium deBaryanum* is the primary parasite involved in root-rot of corn, followed by *Pythium graminicola, Gibberella saubinettii, Rhizoctonia solani, Helminthosporium sativum, etc.* (140); hence this rot is reported as the *Pythium* root-rot of corn. Respecting root-rots of strawberry and tobacco seedlings, the sequence of fungi involved varied with the soil (138). For instance, in muck soil strawberry roots became infected with *Thielaviopsis basicola* in 18 to 24 hours, whereas tobacco roots did not show the fungus until the fourth day following germination of the seed. In strawberry, however, no necrosis took place, nor did infection spread beyond the incipient stage. Following this incipient infection in muck soil, *Pythium* sp., *R. solani,* the Phycomycetous mycorrhizal fungus, orchid rhizoctonia, nematodes and *Asterocystis* were observed in tobacco roots, while *R. solani,* Phycomycetous mycorrhizal fungus, orchid rhizoctonia and *Pythium* species were in strawberry roots in the order given. In a strawberry root-rot soil, however, the sequence was as follows: in tobacco roots—orchid rhizoctonia, nematodes, *R. solani, Pythium* spp., phycomycetous mycorrhizal fungi, *Asterocystis;* in strawberry roots—*R. solani,* nematodes, orchid rhizoctonia, *Pythium,* phycomycetous mycorrhizal fungus, *Asterocystis.* In this connection it is most interesting to note that of the many genera and species of fungi present in a soil, only a comparatively few are commonly associated with root-rots of different host plants. As is to be expected in a complex root-rot of this type, in which the fungi concerned are facultative parasites, the sequence of fungi is not necessarily always the same, since the sequence is affected by variations in soil type, soil flora, temperature, moisture, pH reaction, *etc.* For instance, microscopic examination of the roots of chrysanthemums, roses, gladioli and tulips affected with a root-rot complex indicated a changing fungous flora in the roots throughout the season (19).

TRENDS IN RESEARCH

Obviously the earlier researches on root-rots of crop plants were concerned for the most part directly with host parasite relations and control. With the availability of the Cholodny method and the lacto-phenol and similar techniques for direct microscopic examination of roots, considerable research, especially by Canadian workers (18, 138, 174), has been directed to the general flora as found in affected roots. This aspect has greatly increased our knowledge of the natural root flora and has enlarged our concept of "complex root-rots", that is, complex in the sense that several organisms may be associated with certain rots. For instance, even in the case of *Thielaviopsis basicola,* recognized as the causal agent of black-rot of tobacco, other fungi, especially the so-called phycomycetous mycorrhizal fungus, *Rhizoctonia* spp., including *R. solani, Pythium* spp., and nematodes may be associated in the roots with the primary parasite (174). What rôle these organisms play is not known, though there is good reason to believe that they may follow *T. basicola* and thus aid in the later stages of root-rotting. The presence of these fungi actually within root tissue would suggest this. That *T. basicola* alone is capable of producing severe root-rot is well established, but this does not necessarily rule out the possibility that other soil organisms may, under certain conditions, be associated with *T. basicola.* Moreover, this microscopical technique is well adapted to studying the sequence of fungi involved, as indicated in investigations in infection of strawberry and tobacco seedlings (138). Obviously this technique has its limitations in that it does not follow that all the organisms encountered in roots are necessarily parasites, and certainly microscopic examination alone does not indicate the relative importance of the fungi present. The method has, however, opened up a new approach to the study of root-rots.

In support of the evidence obtained from microscopical studies there is that of inoculation studies to the effect that the parasitic activity of *R. solani* was enhanced when it acted in combination with other fungi associated with the root-rot of cotton in the Punjab, namely *Fusarium solani,* two other strains of *Fusarium* and *Helminthosporium* sp. Also, inoculations made with a mixture of *R. solani* and *R. bataticola* gave increased virulence over inoculations made with either of these fungi alone (322). On the other hand, *R. solani* alone caused a more serious root-rot on *Viola* than when associated with *Pythium* spp. (47), and a mixture of nine strains of *Pythium* was less severe in producing a root-rot of strawberries in Louisiana than any one of the strains alone (237). In another connection, "*Diplodia natalensis* Evans and *Colletotrichum gloeosporioides* Penz. inoculated similarly in slight wounds in citrus bark produced much more marked effects than where each was applied alone" (85), and inoculation of *Phytophthora citrophthora* combined with *Fusarium* sp. produced much more rapidly enlarging lesions of *Pythiacystis* gummosis than did the *Phytophthora* alone (86). The Fusarium when introduced alone was unable to advance at all in this wounded bark.

Another trend, an ecological one, has been the study of the mutual relationship of root-rot organisms to their general soil environment, with special emphasis on the rôle of toxins and antagonistic organisms, and the effects of a changing biological equilibrium on the incidence of root-rot. These studies have established that toxins given off by one organism may affect other organisms adversely by antagonism and that the natural biological equilibrium of a soil may be so changed by manuring, cover cropping, fertilization, rotation, *etc.,* as to render the environment unsuitable for certain specific root-rot-producing parasites, thus reducing the rot. Literature on these phases is rapidly accumulating (33, 34, 130, 132, 133, 197, 219, 264, 268, 285, 336).

The present approach to the study of root-rots, therefore, goes much further than mere host-parasite relationship. Rather, it embraces the inter-association of various components, biological, chemical and physical, as they influence the host-parasite relationship in disease. The limits to our progress in this most complex field are conditioned to-day by our lack of knowledge of much that transpires in a soil, and its relationship to the growing plants; also by lack of suitable technique to study certain phases of the problem, especially the biological and chemical phases.

When it is realized that roots grow in a living, dynamic environment of a most complex nature, it becomes obvious that it is no simple matter to assess correctly the many factors that may be associated in the production of root-rot. Moreover, when it is further realized that any influence which upsets the normal biological balance in the soil, may have a profound effect not only on the soil flora in general, and on the parasites in particular, but on the host plant as well, the difficulties involved in the elucidation of the true nature and cause of certain root-rots become increasingly apparent. Hence, investigations on root-rots, especially of the complex type, should incorporate studies of the chemical, physical and biological interrelationships, in a cooperative investigation, including the chemical, agronomic and biological as well as pathological aspects.

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THE INFERIOR OVARY

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INTRODUCTION

"It is well that from time to time there should be a stocktaking—a full appraisment of our botanical generalizations. (A finer technique and the progress of Science generally offer a sufficient inducement for the arraignment anew of current text-book statements before the bar of observation and experiment. Some supposed facts *may* thereby be more frankly established and others modified and others again possibly rejected.) At the present time, however, a cynical onlooker might be tempted to suggest that botanists were unintentially striving to fix more securely the foundations of their science by the Euclidian *reductio ad absurdum* method".

Thus Dr. Parkin is moved to comment in regard to one of the modern theories concerning the nature of the flower in angiosperms (180). Others have called attention to the need for investigating the flower with minds open to the evidence from all sources (2, 7, 132, 146, 164, 167, 276). After attempting to understand the subtleties of some of these interpretations, this writer is far from taking issue with the above. Since one phase of the problem which is in need of clarification is that of the nature of the inferior ovary, this review of the findings and opinions of workers in this field was undertaken in order to provide a foundation for further study. It cannot claim to be complete, for all the literature was not available, and many observations of value, contained in monographs of special families, have certainly escaped the attention of the reviewer. An attempt, however, has been made to summarize the principal papers dealing with the morphology of the epigynous flower and to show how they have influenced the course of thought during the last one hundred and fifty years.

The various theories which have been held in regard to the nature of the flower in general have been so well reviewed (2, 7, 276) that they will be restated only in so far as is necessary for an understanding of this limited phase of the problem. Since the so-called "classic theory", in which the flower is regarded as a short shoot, or a collection of metamorphosized leaves, remained essentially unchallenged from the time of Linnaeus until the late nineteen twenties, most of the research on the inferior ovary has been based upon this assumption. According to the classical theory, the carpel is considered the equivalent of a folded leaf (or sporophyll) which produces ovules on its inturned margins; it is the unit of structure of the gynoecium of all angiospermous flowers, and is appendicular in its nature.

Although credit for originating the appendicular theory is usually given to Linnaeus, it appears doubtful that he had a very clear-cut idea of the carpel and its role in the formation of a compound pistil. He used the term "pistillum" for both simple and compound structures, and distinguished between "germen superum" and "sub receptaculo" or "infra receptaculum". He at first believed that the pistil, the innermost organ, came from the pith, the innermost tissue of the stem, which, being the soul of the plant, passed on its immortality to the seeds (161). Later (162), however, he developed his idea of metamorphosis, in which he postulated that the flower is the equivalent of a bud containing the leaves of six successive years to come, the pistils being the equivalents of the leaves of the sixth year. He considered that the ovules were buds, an idea which persisted in the minds of some morphologists until about 1880, and was largely responsible for the die-hard theory that these seed-buds could be produced only on an axis. Linnaeus, however, did recognize cohesion and adnation between floral members.

Caspar Wolff (278), in his "Theoria generationis" of 1759, after stating that in the plant whose parts seemed at first so extraordinarily diverse he recognized only stem and leaves, included the seed capsule with its seeds in the latter category. One could see this, he added, when the ripe capsule broke apart and split into leaves which had previously been joined together. Unruh (253) considers that Wolff gave us our first definition of the carpel, but the term "carpelle" seems first to have been used by the de Candolles (53). Goethe (103, 68), who worked out his theory of metamorphosis independently, went but little further. According to Arber (2), "He recognized the legume equal to a single folded leaf, concrescent at its margins and bearing ovules which he regarded as buds of the next generation. He was also clear about that type of syncarpous gynaeceum in which the 'carpellary leaves' are united edge to edge, but he had not enough botanical knowledge to develop his views and it was A. P. de Candolle (1827) who put the foliar interpretation on a firmer basis".

Not only did A. P. de Candolle give us a concept of the ideal flower which he considered to be polypetalous, hypogynous and radially symmetrical, as variants of which all others were created, but he considered that the causes which were responsible for the divergence of flowers from the original type were abortion, degeneration and adherence of parts (49). In his "Prodronus" (52) he emphasized the fact that the whole art of classification consisted in discovering the plan of symmetry and in making abstraction of all the deviations from it. His method of discovering the original plan when members were lost consisted in making studies of monstrosities and comparisons of analogous parts. How close he came to going beyond the dogma of the constancy of species without realizing it was the subject of an amusing comment by Sachs (200), who added that this dogma, "as Lange wittily remarked, 'comes direct from Noah's ark' ".

Granted that the concept of the carpel as a folded leaf (or even a folded sporophyll), the stumbling block of the "new morphologists", is a too literal interpretation of the gynoecial unit, if one accepts the Lignier hypothesis of the origin of angiosperms from fern-like ancestors derived from dichotomously-branched algae, the term "carpel" is a too convenient one to discard. One can still use it in a descriptive sense, without implying that the organ indicated reached the stage of a full-fledged foliage leaf before it folded and became a carpel. The venerable Dr. Scott, however, was not troubled by the use of the term "leaf". He said (222): "In Pteridosperms the reproductive organs are borne on appendages already fully differentiated as leaves. No doubt both the fertile and sterile portions were once thallus-branches, but in Pteridosperms they have obviously become *foliar*. To call the fertile portions branches or branch systems seems an anachronism! They were no longer that at the Pteridosperm stage". Most morphologists accept Mrs. Arber's suggestion (2) that the carpel may be considered an appendicular organ which has undergone a development *parallel* with that of the vegetative leaf; in other words, it is a leaf-like structure, essentially a phyllome. From the work of Sinnott and Bailey (226) and Eames (83) we may obtain a concept of this primitive structure. Comparative studies of existing pistils led them to the conclusion that it was a palmately-lobed appendage, containing a median (dorsal) and two lateral (ventral) traces, which left gaps in the axial stele (*Fig.* 7). From this a second type, scarcely more advanced and containing in addition two median-lateral veins, was probably derived (*Fig.* 8).

Hunt (128) interpreted these simple carpels as derivatives of branch systems, and added: "In a sense axis, leaf, and carpel represent distal portions of the branch system in contrast to the stem, which is a sympodial arrangement of the proximal part". Wilson in 1937 decided that the stamen had a similar origin and recently supported his contention with additional evidence (273, 274). In his later paper he accounted for the carpel also on the basis of the telome theory, suggesting that it owed its origin to "the flattening, webbing, and fusion of fertile branches, which folded along their margins". He was careful to state, however, that this conception of stamen and carpel did not invalidate our generally held view of the flower as an axis with highly specialized leaf-like appendages, since the leaves, themselves, have evolved in a similar way.

THEORIES CONCERNING THE NATURE OF THE INFERIOR OVARY

During the century that followed de Candolle's statement of the doctrine of symmetry, several theories with many modifications have been current regarding the nature of the inferior ovary. The most important are as follows: (*a*) it is the result of concrescence of all the floral whorls (*Fig.* 1); (*b*) it is entirely axial, excepting for its apical covering (*Fig.* 3); (*c*) it consists of an axial cup lined on the inside with carpels (*Fig.* 4); (*d*) it consists of a hollow axis containing a carpellary placenta (*Fig.* 6); (*e*) it is a structure "sui generis"; (*f*) it is a sporogenous axis in which toral growth dominates apical growth.

(*a*) *Concrescence of floral whorls* (*Figs.* 1, 2). This is frequently referred to as the de Candollian theory, since it was first clearly formulated by A. P. de Candolle. Later it became connected with the name of Van Tieghem, because of his strong championship of it. Since it postulates that the inferior ovary has been developed through complete coalescence of the bases of corolla, stamens and carpels, the ovary is appendicular, except for a possible penetration of the axis in the center of the flower as a bearer of carpels. The theory was the current one of the early nineteenth century. From about 1840, it lost ground in competition with theories *b* and *c,* but in the eighteen-seventies was revived by Van Tieghem and gained many adherents, particularly in France and England. At the end of the century it suffered another eclipse which lasted until about 1920, when it was again supported by Eames and his students as the explanation of the nature of the great majority of inferior ovaries.

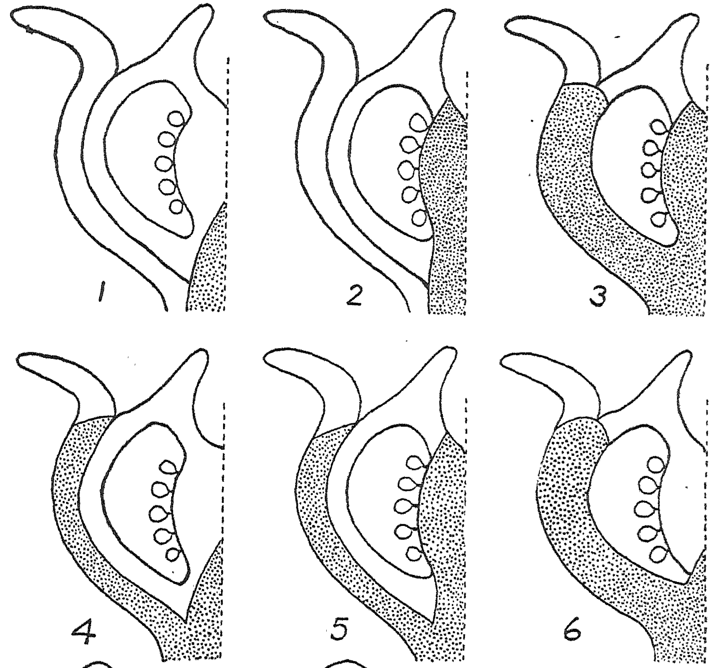
(*b*) *Modified axis* (*Fig.* 3). According to this theory the inferior ovary consists largely, if not entirely, of receptacle tissue modified for reproduction. Carpels are borne on its upper rim, but these function merely as sterile coverings which roof over the ovarian cavity or are reduced to styles and stigmas. The theory originated with Schleiden, was adopted with some modifications by Payer, Hofmeister, and Sachs, and was held by most German and a few French and English botanists until it was gradually superseded by the following. The recently proposed theory of Hagerup is a variant of the Schleiden hypothesis.

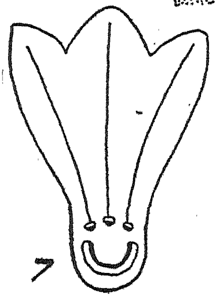
(*c*) *Concave receptacle, lined with carpels* (*Figs.* 4, 5). According to this theory the tip of the peduncle during development becomes invaginated and fused with the carpels which are produced on its inner surface. Sepals, petals and stamens are borne on its outer rim. The resulting inferior ovary, therefore, is not different from the superior one, excepting for its outer axial cup. The authorship of this theory is often attributed to Goebel, or Čelakovský, but, as Vidal (264) rightly points out, it owes its paternity to Naudin (177) and Decaisne (71). Duchartre (79) also held that the ovary of *Oenothera* developed within an invaginated axis, but he considered that the placenta was axial (*Fig.* 5). After the hollow-axis-carpellary theory had rallied to its support such "top-notch" botanists as Čelakovský, Warming and Goebel in the eighteen seventies and eighties, it came into general acceptance in Germany and gradually gained in popularity in other countries until at the turn of the century it became the dominant theory.

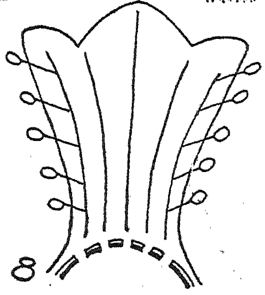
(*d*) *Cup entirely axial, placenta carpellary.* (*Fig.* 6). Sachs (197, 198) believed that the wall of the true epigynous flower was axial in all cases, but that the placenta could be either axial or carpellary. When carpellary, he thought that it originated by downward extension of the carpellary margins on the inside of the ovary wall; in unilocular inferior ovaries these margins formed the parietal placentae; in multilocular ones they were responsible for the dissepiments as well, which could either meet in the center and produce ovules in the angles between them or turn back toward the margin before doing so. Bugnon (42, 43) is the chief modern advocate of this theory. He explains the displacements of the floral members on the basis of inequality in growth of the apical and peripheral regions of the floral axis.

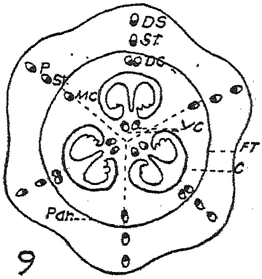
(*e*) *Inferior ovary a structure "sui generis".* Several botanists, among them Ganong (93) and Grégoire (109, 110, 111), have held the opinion that, irrespective of its origin, the inferior ovary should be considered as a new structure which has acquired an identity and character of its own, and that it is futile to attempt to look for axial and appendicular parts within it. In general, this represents the position taken by J. M. Coulter and the ontogenetic school, who explain the epigynous flower on the basis of what is observed during development of the bud, namely, formation of a cup-like depression in the center of the receptacle because of intercalary growth taking place *en masse* at its rim, thus elevating the carpels, stamens, petals and sepals to a position on top of the ovary. This view is certainly reminiscent of that of Schleiden, except for insistence on the fact that the resulting structure is a new one which attains the full status of an organ. Grégoire, in proposing his "sui generis" theory, advanced the idea that the flower should be regarded as a transitory meristem-carrier, implanted on a permanent vegetative cone, which is responsible for the development of reproductive organs and is in no way homologous with a modified vegetative shoot.

(*f*) *The inferior ovary a sporogenous axis in which toral growth dominates apical growth.* This is the view of J. McLean Thompson (239) who looks upon the flower as a sporogenous axis in which toral and apical growth is taking place. Dominance of the former over the latter results in the production of an inferior, and of the latter over the former a superior ovary.









Figs. 1–6. Diagrams representing types of inferior ovaries with varying amounts of axial (shaded) and appendicular (unshaded) tissues—modified from Bugnon (42); fig. 1, ovary entirely appendicular; 2, ovary appendicular except for axial placenta; 3, ovary axial except for covering; 4, cup axial, enclosing an appendicular ovary; 5, cup axial, enclosing an appendicular ovary with an axial placenta; 6, wall axial, placenta appendicular. Fig. 7, Sinnott and Bailey's primitive carpel (226). Fig. 8, Eames' more advanced carpel (83). Fig. 9, Cross section of flower bud of *Alstroemeria*, showing a floral tube, in which all bundles are distinct, fused to a tri-carpellate ovary—after Van Tieghem (258). FT indicates floral tube; C, carpel; Par., parenchyma band; DS, St., P, DC, MC, VC, dorsal sepal, stamen, petal, dorsal carpel, median carpel, ventral carpel traces, respectively.

Modifications of these basic theories were frequent. Differences of opinion arose over the presence or absence of the axis in the center of an appendicular ovary (*Figs.* 1, 2), or over the amount of carpellary tissue entering into an ovary developing out of an invaginated receptacle (*Figs.* 3, 4, 5, 6). Also, complete accord with respect to the origin of the carpel has never been attained. Occasionally it was conceded that inferior ovaries might have arisen in different ways in different plants. Generally, however, each advocate of a theory vigorously defended his own and sharply criticised those of his opponents. Perhaps no phase of plant morphology has been the subject of more bitter controversy than this.

METHODS OF STUDY

The methods which have been employed from time to time in investigation of the problem involve: descriptive morphology, comparative morphology, ontogeny, histology, teratology, vascular anatomy, comparative anatomy and paleobotany. Much of the controversy has arisen because many investigators have made use of the ontogenetic and teratological bases for evidence in support of their theories to the exclusion of the anatomical and the comparative, even in recent times when conclusions reached by employment of the two latter have proven to be of prime importance in other groups, such as the ferns and gymnosperms, as well as in animals. Zoologists, the writer understands, are using great caution in drawing conclusions from developmental evidence alone. In plants this evidence seems to be inconclusive. Velenovský (262), in emphasizing the value of the comparative method for solving morphological problems, stated that all data derived from young stages of organs should be discarded. Teratological data, also, are often so conflicting that they may be interpreted either *pro* or *con,* and most modern investigators are inclined to use them as contributory rather than basic for drawing conclusions. Mrs. Arber (2), in proposing that they be used in this way, made this discerning comment: "Though there is no reason to suppose that abnormalities provide information about ancestral conditions, it is an undeniable truth—indeed a truism—that abnormal forms can show what an organism *can do*".

Some morphologists, including Thompson (239), Goebel (102) and Grégoire (109, 110, 111), have discounted the data derived from vascular anatomy, claiming, in accordance with a general rule offered by Grélot (112) in 1897, that a vascular bundle always belongs to or is intended for an organ externally expressed and retaining its ground tissue, that the whole doctrine of the conservativeness of the vascular skeleton should be discarded. This, however, seems contrary to the findings of most students of both angiosperms and gymnosperms, who agree with Kozo-Poljansky's assertion: "this has never been proved nor is it generally valid" (146). With respect to anatomy, Eames (83), who makes use of it in a comparative way, made the following statement: "It does not claim to provide the only evidence as to the fundamental morphology of the flower: it claims only that its evidence is of the very best, because of the conservation of vascular tissues under evolutionary modification. The determination of fundamental structures must rest ultimately upon facts provided by taxonomy, descriptive and comparative morphology, paleobotany and ontogeny". Certainly no morphologist who has witnessed the great advance in our knowledge of structure and relationships among the lower vascular plants by employment of the comparative anatomical method should question its use when applied to angiosperms.

EARLY OBSERVATIONS

According to Greene (108), the different types of insertion of floral members characteristic of hypogynous, perigynous and epigynous plants were clearly recognized by Theophrastus. Greene translates Theophrastus as follows: "Some produce the flower around the [base of the] fruit, as do the grape vine and olive tree. . . . In the greater proportion of plants the fruit thus occupies the center of the flower. But there are not wanting such as support the flower on the summit of the fruit, as do the pomegranate, apple, and rose, all of which have their seeds [ovules] underneath the flower. A few bear the flower on the summit of the seed itself, such as thistles, and all that have their flowers in that manner crowded together". Greene states further that Theophrastus "learned this springing of the 'flower' from the top of the 'seed' to be characteristic of the whole family of umbellifers, and a few of the rubiaceous plants that he knew, as well as of the thistles and their kindred. . . . At this juncture the sublime old Greek will appear to have lived before his time by more than two thousand years".

Treviranus (246) credited Tournefort with the proposal that the inferior ovary represents a fusion of the calyx tube to the ovary. The terms "stamena hypogyna," "perigyna" and "epigyna" were first employed by A. L. de Jussieu (139) in 1789, who used them as criteria for sub-dividing his primary groups within the monocotyledons and dicotyledons. Mirbel (173, 174) divided pistils into two groups which he characterized as "adherens" or "demi-adherens" on the basis of the amount of adherence of their ovaries with their perianths.

In the first two editions of the "Théorie élémentaire" (47, 48), de Candolle explained the position of the petals and stamens on the top of the inferior ovary as due to the adherence of the calyx tube to the ovary wall, as had Mirbel. By 1827, however, he had come to realize that it was necessary to explain further how this elevated position had been attained. In his "Organographie Végétal" (49) he suggested the following alternatives: either the bases of the middle whorls (petals and stamens) were included in a common fusion of all the cycles, or their free portions were borne aloft by an intermediate structure which cemented the calyx-tube and ovary wall together. He inclined toward the latter explanation and adopted Salisbury's term, "torus", which the latter author, however, had defined as "the common support, or base, of the different parts of a simple flower" (202), for this soldering layer. De Candolle stated that he did not consider this uniting zone to be deserving of the status of an organ, inasmuch as it probably represented only the very much reduced bases of the stamens and petals. The semi-inferior ovary was explained by him in the same way, as the result of the adherence of the torus, in the form of a circular ring, to the bases of the calyx and ovary.

There has been a great deal of confusion in the use of the word "torus". Achille Richard, whose textbooks ran through many editions between 1819 and 1876, stated in 1838 (191) that he disagreed with the author of "Organographie Végétal" in his use of the term, which was commonly considered to refer to the summit of the peduncle and the point of origin of the constituent parts of the flower. He thought that de Candolle had confused the torus with the disk, which most botanists did regard as an independent and appendicular organ. Bischoff (23), also, wrote in his textbook of 1834 that the swelling from which the corolla and androecium arises should not be looked upon as an independent torus, "as many in modern times were doing", but as a fusion of corolla and androecium, which covers the inner calyx-tube surface up to the point where the sepals become free. Alphonse de Candolle (46), after presenting the alternative suggestions of his father, said that the toral explanation seemed to him the more natural for most cases, but that there were some about which it would be embarassing to say whether the fruit were made up of a prolongation of the torus or consisted of the united bases of stamens and petals. There was little uniformity in the use of the term by later writers. Some looked upon it as elevated receptacle, or thalamus, some as disk, and some as the attentuated base of the stamens and petals. Clos (62) in 1854 suggested that because of the ambiguity in the meaning of the term, "torus", it should be abandoned, and Linnaeus' well-understood "receptacle" be re-instated when referring to the top of the peduncle. Arthur Henfry's textbook of 1870 (121) contains this sentence: "This structure [torus] is the most frequent bond of union between cohering whorls, and in such cases we find calyx, corolla, stamens, and carpels not directly in contact but arising from the torus, in the substance of which their inferior extremities are lost".

Mrs. Hooker (155) translated Le Maout and Decaisne's definition as follows: "The torus is the part of the receptacle situated between the calyx and pistil on which the corolla and androecium are inserted. It is merely the periphery of the receptacle and not a special organ: but for convenience it is so considered". Later the term came again to include the whole of the top of the peduncle. With the exception of this slight difference of opinion in regard to the torus, apparently all early botanists until about 1840 followed de Candolle in considering the inferior ovary to be a structure resulting from the coalescence of floral members.

SCHLEIDEN AND THE INTRODUCTION OF ONTOGENETIC RESEARCH

In 1837 came the first dissenting voice. It was Schleiden's—and how it did dissent! He began by taking to task those who in seeking high authority in the study of nature neglect the only high authority, nature herself. After having made a comparative study of the Coniferae and the Angiospermae, he had become thoroughly convinced of the truth of the theory that ovules can be borne only

on an axis (216). "Betrachten wir den ganzen Complex der Pflanzenwelt, so finden wir es als durchgreifendes Gesetz, dass sich niemals eine Knospe an einen Blatte bildet, sondern nur an der Axe und den von ihr abgeleiteten Organen. Sieht man nun die Ovula als Knospen an, so hätte man auch consequent weiter schleissen müssen, dass die Placenta eine umgebildete Axe sei. Was hat man aber, um diese einfache und notwendige Folgerung umzuwerfen angeführt". In reply to those critics who embarassingly brought up the case of *Bryophyllum, Malaxis* or *Ornithogalum,* he explained that the so-called "leaf" of *Bryophyllum* was in reality a stem, and since buds on the leaves of the others were abnormalities anyway, they were of little significance.

Two years later (217), as the result of a comparative study of developing placentae, he reached the conclusion that the true inferior ovary was not formed by carpellary leaves but simply by invagination of the axis, as in *Ficus.* In support of this point of view he cited the fact that the ovarian cavity in developing buds is completely formed previous to or simultaneously with the appearance of carpel fundaments and external whorls; also he repeated his former contention that ovules could never be produced on other than axial structures. He considered that the ovule integuments were also cauline. In his textbook (219, 220) he insisted that the history of development alone should be the guide in the interpretation of floral structures, and went even further in making the following suggestion regarding the superior ovary (220):

"When a conviction has been arrived at of the correctness of the preceding exposition, . . . the notion will be more readily accepted that the superior pistil may also be wholly composed of axial organs. The following axioms will serve as a base of departure: axis and leaf are not distinguishable by any difference of external form, but by their peculiar processes of development; in the leaf the apex is formed first, the base last; in the axis the contrary is the universal rule. That which regularly produces normal buds is not a leaf but an axial organ".

Regarding the doctrine of metamorphosis, he added: "It is in fact no other than an isolated, fragmentary application of the only really scientific principle which botany can at present possess: namely that of progressive development"—and in conclusion, "Little can be expected of anatomy".

Logical deductions from these premises led him to the conclusion that the inferior ovary consisted of a hollow receptacle (disk), formed by an extended growth of the internodes between the floral verticels. The carpels furnished more or less extensive coverings for the ovaries of the families Saxifragaceae and Myrtaceae, but were reduced to styles and stigmas in the Onagraceae. He defined the disk as any expansion of the internodes of the flower which did not immediately bear seed-buds. Reasoning further he came to the strange conclusion that the "stem-pistil" of the Leguminosae and Liliaceae was developed out of flat, leaf-like structures which were really cauline. In spite of his wishful search for axial walls in all ovaries, he was forced to admit that the walls of "true" superior ovaries and of the carpels of rosaceous genera, other than *Pyrus,* were foliar. He considered that the placentae ("spermatophores") of superior ovaries, however, represented forking extensions of the axis which overlaid the carpel margins.

Although Schleiden's opinions were based on false premises, Sachs (199, 200) felt that he had made a most valuable contribution to botanical science by his stimulation of research on the genesis of the flower, and by his introduction of a new method. His views were accepted by Endlicher and Unger (87) and other German botanists rather generally, but never met with universal acceptance in France and England. Achille Richard, an adherent of the appendicular school, was apparently influenced by Schleiden's concept of the placenta. In the sixth edition of his textbook, published in 1838 (191), he described the "trophosperme" as a delicate and attenuate structure, which, originating in the stem where the peduncle gives rise to the carpel, adhered to the suture of the carpel and produced the ovules. He regarded it as double, with each fork applied to each edge of the carpellary leaf. Later Richard (192), influenced by the work of Brongniard on floral monstrosities, decided that in certain cases the placentae were appendicular, and in subsequent editions dropped the idea of the axial trophosperme. August de Saint-Hilaire (201) was the first French botanist to adopt the Schleiden hypothesis in its entirety. He looked upon the wall as receptacular cup, the placenta as an axial "columella" containing the ovule-supplying bundles ("cordons pistillaires"), which arose in pairs from the axils of sterile carpellary leaves (dissepiments), and the ovules as miniature branches. Regarding the calyx-tube of the Rosaceae he argued as follows: "The calyx tube is an aggregation of leaves. A leaf can never be borne on another leaf, so that it is necessary to see here something other than calyx. What can it be but receptacle?".

Most botanists of the appendicular school, who believed that the wall represented a fusion of the bases of all the floral whorls, thought that the placenta might be axial in certain cases (*Fig.* 2). In John Lindley's "Elements of Botany" (159) we find the following: "But although the placenta of many plants appears to derive its origin from the margin of the carpels, it is certain that in many cases the placenta is merely a development of the center of the flower-bud and is in reality the end of the medullary system". Alexander Braun,—to whom floral morphology is indebted for the idea that the basic number of carpel whorls in the flower is two, and that sometimes the outer, and sometimes the inner is suppressed (32),—believed that the placenta was an integral part of the carpel, although he admitted that in certain plants, characterized by free-central placentae, there were grounds for considering that the ovules were axis-borne (33). Adolph Brongniart (34), although he took issue with Schleiden concerning universal development of ovules from an axis, thought that this was the case in the Primulaceae, Myrsinaceae, Theophrastaceae and possibly in the Santalaceae. Adrien de Jussieu (138) also, while subscribing to the appendicular theory for flowers in general, made an exception of the rose, in which the carpels seemed to be developed on a depressed torus.

EMERGENCE OF THE AXIAL-CUP-CARPELLARY THEORY

Schleiden's work came to the attention of Duchartre who up to this time had accepted the view of de Candolle and had explained the ovary of *Dipsacus* and *Helianthus* as a calyx-tube fused to the ovary (78). He made use of the ontogenetic method in studying the bud of *Oenothera suaveolens* (79) and came to the conclusion that the inferior ovary of this form resulted from growth of four carpels within a hollowed receptacle, which produced at its summit the sepals, petals and stamens.

Within the ovarian cavity the inward-turning carpellary walls formed the four partitions, which at first united but were afterwards separated by the penetration of a central axial columella, upon which were produced the ovules (*Fig.* 5). His view of the placenta was similar to that of Schleiden, but his view of the double nature of the wall was new. He differed from Schleiden, also, in considering that the dissepiments were foliar. Duchartre was apparently the first investigator making use of the ontogenetic method to attach any significance to vascular anatomy. In 1875 Van Tieghem (258) wrote that Duchartre held a theory similar to that of Schleiden, but was more prudent in not insisting on the universality of it. Duchartre had stated in his textbook (80) that it was customary to consider that the inferior ovary represented an adherence of the floral verticels, but that this was not always so could be seen by an examination of *Cucumis Melo,* in which the outside wall was peduncle, and of *Scabiosa,* in which it was involucre. In 1891 (81) he concluded that the outer wall of a pomaceous fruit, which encloses a carpellary ovary, consists of peduncle tissue, since abnormal specimens often produce floral parts on their surfaces. Duchartre was especially interested in teratology, and his collection of monstrosities was the object of much interest and comment at the time.

Duchartre's work on *Oenothera* was followed by Trécul's on the campanulacean genus *Prismatocarpus* (242). Trécul stated that his results confirmed those of Duchartre, but his conception of the fruit seems to be closer to that of Schleiden, inasmuch as he considered it an axis modified for reproduction. Trécul, whose major interest was vascular anatomy, said that he saw little correspondence between the course of the bundles in the fruits of *P. Speculum* and *P. hybridum* and a branch with a 2/5 phyllotaxy. Yet he did note in the longitudinal sections of the flower nine bundles in groups of three, supplying the carpels, and five entering each member of the stamen, petal and sepal whorls, while two branches of the remainder continued up in the center of the flower and produced the "cordons pistillaires". He reasoned that the summit of the ovary must represent the axis, since it gave rise to the floral members. Buchenau (40), as a result of an ontogenetic study of the Compositae and Umbelliferae, and Chatin (58), after investigating *Vallisneria spiralis,* in which he noted that the cavity is a late development, also applied the Schleiden explanation to the inferior ovaries of these forms.

On the other hand, Barnéould (9) attributed the inferiority of the ovary of *Trapa natans* to the fusion of two basally-united carpels to the calyx and corolla tube (whose segments arise united) by means of a circular disk which grows up from the receptacle in the region between them. Lestiboudois (157), after studying the course of bundles in a number of genera, including *Ricinus, Narcissus, Gladiolus* and certain species of the Umbelliferae, as well as *Oenothera,* rejected the ideas of Duchartre and Trécul. He decided that the floral organs were analogous to leaves and that the course of their bundles was normal in every case, since they exhibited the same underlying principles of phyllotaxy which he had noted in an earlier study (156) to be characteristic of appendages. The two-layered fruit-wall of *Oenothera* was explained as pericarp fused to a tube made up of calyx, corolla and androecium bases. He suggested that the primitive carpel was similar to that of *Soya,* in which the vascular supply consisted of a median vein, two marginals, supplying the placenta, and often two median-laterals as well. Regarding the "central body" of Duchartre, he said that this so-called columella was "no stranger to the leaf", inasmuch as it represented a fusion of the marginal bundles.

In 1855 Naudin (177) made a comparative study of the leaf and flower of the Cucurbitaceae and decided that the inferior ovary of this family consisted of a hollowed-out axis containing an appendicular compound ovary (*Fig.* 4). His paper was the first to suggest that the ovary developing within the hollow cup was entirely foliar, and to insist that superior and inferior ovaries, built upon the same plan, were essentially alike and differed only in the nature of the outer wall. His conclusions, obtained from a study of abnormal forms and of developing buds, are summed up in the following paragraph:

"There is no adherence of the calyx to the wall of the ovary, which botanists up to the present time have held. This hypothesis, which is not supported by facts, and which we have opposed, has been current since the time of Linnaeus, and for a long time has been accepted without question. I would not absolutely deny that walls resulting from fusions do not exist in the plant kingdom; but I do not know of any instance where the inferior ovary cannot be better explained by the sinking of the ovary in the peduncle of the flower" [Translation].

Two years later, Decaisne (71), whose original diagram of the pear fruit appeared in many later textbooks, interpreted the pome as Naudin had the melon. He decided, after making an ontogenetic study, that the outer flesh was receptacular; also that during early growth the carpels became cemented together and to the cup by the development of a delicate new tissue, originating in the disk and distinguishable in the fruit by its granulations. He compared the pome with the fruit of *Anacardium* and *Hovenia,* and reached the conclusion that all inferior ovaries developed in the same way. The idea that the fruit of the pear consists of a hollow axis containing free carpels was not new. It goes back to Malpighi's illustration in the "Anatome plantarum" (168). Treviranus (246), who in 1859 applied this explanation to the fruit of mistletoe, reported that it had been held by Caspary and Duhamel also.

In the same year, 1857, Payer's "Éléments de Botanique" (183) as well as his famous "Traité d' Organogénie Comparée de la Fleur" (184) also appeared. His view of the morphological nature of the pistil was expressed as follows (183):

"Toute pistil se compose d'un *partie axile,* qui est formée par l'extrémité du réceptacle, et d'*un ou plusieurs organes appendiculaires,* analogues aux feuilles, et qu'on nomme, pour cette raison, *feuilles carpillaires.* Sa partie axile constitue les placentas et porte les ovules; les feuilles carpellaires constituent les parois de l'ovaire".

"Dans tous les pistils à ovaire supère, les parois de l'ovaire sont formées en totalité, comme dans le Mouron rouge, les *Celosia* etc., ou en grand partie, comme dans la Violette, l'*Hypericum* par les feuilles carpellaires, c'est-à-dire par la partie appendiculaire du pistil. Dans tous les pistils à ovaire infère, au contraire, la plus grand partie des parois de l'ovaire est formée par les bords du réceptacle que s'est évasé, et par conséquent, par la partie axile".

With these basic ideas in mind he followed the development of the flowers of a great many families, and his "Traité", abundantly documented and beautifully illustrated, became the standard work on ontogeny, and one which stimulated many others to take up research in that field. Occasionally Payer's axioms led him to some strange explanations. For example, the ovary of *Myrtus* was described as consisting of two parts, entirely different in their origin. The lower axial region contained the placenta and four cavities which developed in the receptacle; the upper carpellary portion furnished the cover and the parietal placentae which extended to the center. The placentae, themselves, were considered to be sterile, but each unit was overlaid by two branches of a forked axis (Schleiden's suggestion). To Van Tieghem, with his more simple explanation, applicable to all cases, this seemed an absurdity, and he became Payer's chief critic.

Schacht (211) resolved the problem thus: The pistil can be considered to be a structure built up of carpels; but it can also be carpellary in its upper part and consist of a hollow receptacle below; it can even be made up of hollow stem entirely. The superior ovary can truly be considered as foliar, whereas the inferior would appear in every case to be a hollow stem. He and other textbook writers of the period, including Treviranus (245), agreed with Decaisne (71) in considering the pome as a hollow perigynous receptacle containing free carpels which were later cemented together and with the cup by tissue growth. To this idea Karsten (140) delivered the coup de grâce by showing that during development of the bud the carpels are never free but are involved in a common growth with the receptacle. He concluded, as had Naudin and Decaisne, that the core of the pome was a carpellary structure.

RESURGENCE OF THE APPENDICULAR THEORY

Von Mohl (175), after having made a microscopic study of the carpophore of the Umbelliferae, in which he compared the vascular anatomy of several species, decided that it was entirely foliar. Furthermore, he insisted that nature always follows a uniform plan in the building of a pericarp. There was also Van Tieghem to be reckoned with. In 1868 the first part of his mémoire, "Recherches sur la structure du pistil, et sur l'anatomie comparée de la fleur", for which he had been awarded the Bordin prize by the Académie des Sciences, appeared in the Annales des Science (254). As an uncompromizing champion of the appendicular theory, he became the outstanding opponent of Schleiden and Payer. To Van Tieghem, who for a number of years had carried on extensive comparative and anatomical studies of the flowers of many families, there appeared to be no difference between the superior, semi-inferior and inferior ovaries, except in the degree of fusion involving their floral members. The inferior ovary represented "la somme des appendices"—sepals, petals, stamens and carpels. He insisted that the real point of insertion of a floral member on an axis was the point at which the vascular bundles left the stele, rather than the point at which the organs became free. This position might be above or below the apparent insertion, because the bundles of the two systems often remain together in the "receptacular tube". Often the single common bundles of the tube represent fusions of the traces of all the outer whorls, which become free at the top of the tube. Thus they could not be considered axial, as Trécul had thought. He argued that if one wished to consider the so-called receptacular tube of *Spiraea* as axial, he must consider that the corolla tube of the primrose and plumbago belong in the same category. Since the placentae of these flowers were considered to be axial, one would have to visualise an encasement of the axis by the axis—clearly an impossibility. He was ready to admit, however, that in certain flowers, such as *Rhododendron,* the axis might penetrate the center of the flower, functioning as a bearer of the carpels. When present, this extension of the peduncle was called a "transitory axis".

This paper, with the incorporation of his researches on the comparative anatomy of the flower, was published again in 1871 (257) and further elaborated into his exhaustive memoir of 1875 (258) embodying studies of a large number of genera of important families. In every case he interpreted the epigynous ovary as a structure resulting from coalescence of floral verticels, and for that reason he considered that it was improperly named inferior. One of Van Tieghem's most convincing lines of evidence was the application of the fact that whenever placental bundles destined for the ovules become inverted, they should be considered as marginal carpellary, rather than as axial traces. In an inferior ovary these marginal carpellary bundles leave the stele at the base of the ovary, while the bundles of the carpel midribs, stamens, petals and sepals often remain united until they reach the top of the ovary. He insisted that these common bundles should be regarded as foliar, rather than as stelar. The genus *Samolus,* figured by many authors as an example of an inferior ovary with a free central placenta, shows very clearly these wall bundles, as well as their tangential separation at the top of the ovary (*Fig.* 17). Recent work (74, 75) has shown that the placenta of this genus is largely, if not entirely, carpellary, since it has become "free" by the detaching of the carpel margins, containing ventral inversely-oriented bundles, from their dorsal parts—precisely as in superior-ovaried forms of the same family. Van Tieghem (256) looked upon this as a structure built up of the "tallons" (soles) of the carpellary leaves.

The year 1868 was memorable also because of the publication of Hofmeister's "Allgemeine Morphologie der Gewächse" (126). His developmental studies threw the weight of his great authority to the axial theory of Schleiden. The fact that during the ontogeny of the flower the hollow cavity in the tip of the peduncle appears with or before the primordia of the appendages, while the outer wall becomes elongated through intercalary growth, was the basis of his reasoning, as it was of Schleiden's and the other ontogenists. Hofmeister, however, thought that the carpel walls took some part in the formation of the septa.

In view of the opinion prevalent in Germany around 1869, it was remarkable that Emil Koehne (145) explained the ovary of the Compositae as a product of growth of the calyx-corolla-stamen-tube to the carpel. Koehne made use of vascular anatomy as well as ontogeny in his study. Since he found traces of all the floral whorls in the common parenchyma of the wall, he decided that it must be entirely appendicular. He looked upon the disk as a swollen appendage of the base of the style. In the same year Cave (56) presented an important paper, dealing with the structure and development of the fruit, in which the opinions of some of his contemporaries may be found. According to Cave, most competent botanists of the time, following Trécul and Duchartre, rejected the Van Tieghem theory of concrescence in respect to the inferior ovary, although many of them believed that the superior ovary was appendicular. Much difference of opinion prevailed over how much of the axis was present in the epigynous flower. Cave said that he himself was in substantial agreement with Van Tieghem, but he thought that the receptacular tube of the pome fruit and rose showed the structure of a stem. He added that most of the eminent botanists, including Robert Brown, Brongniart, Duchartre, Naudin and Decaisne, felt that Van Tieghem had failed to distinguish between the ovary wall and the receptacular tube to which it was united. The writer has, however, been unable to find any statement by Robert Brown which dissented from Van Tieghem's point of view. In his textbook Brown (37) explained the inferior ovary on the basis of the coalescence of the lower portions of the outer floral verticels with the ovary wall.

Sachs (197, 198) ruled out the possibility of the inferior ovary originating by cohesion and adnation of floral members. He believed, further, that the tube of the Sympetalae was receptacular. He made the distinction between the true inferior ovary, characteristic of epigynous flowers, whose wall was axial, and the inferior ovary of perigynous flowers, which was equivalent to a superior ovary buried in the receptacle. In the true inferior ovary the wall resulted from the more vigorous growth of the torus at its periphery than at its center, as the result of which the free carpels, together with the stamens and petals, were carried up on its rim, where they finally closed the opening and formed the styles and stigmas. He recognized the same type of placentation in the inferior as in the superior ovary, and called attention to the numerous semi-inferior-ovaried forms which were intermediate between the two. The parietal placentae of the unilocular ovary were interpreted as prolonged carpel margins, extending downward along the wall: dissepiments and placentae of the multilocular type were similarly explained. In both these cases the ovules would, of course, be carpel-borne. He thought, however, that they were axis-borne in those cases in which the tip of the peduncle penetrated the center of the flower and produced terminal (as in *Rheum, Najas* or *Typha*) or lateral ovules. In the latter case the ovules might be borne on either a free-central (as in *Samolus*) or an arrested placenta (as in *Helianthus*). These criteria were used in his classification of both superior and inferior ovaries. Sachs' views were widely adopted. Warming (266) was exceptional in continuing firm in his belief that the placenta always belonged to the carpel, and Schenk's textbook of 1879 (215) surprisingly defined the inferior ovary as a structure resulting from fusion of perianth and ovary.

The changing opinion of the time is reflected in La Maout and Decaisne's textbook of 1868 (154). After a discussion of the different points of view regarding the inferior ovary, this sentence appears in the English edition (155): "Hence what has hitherto been called adherent calyx ought to be called receptacular tube or cup". La Maout's textbooks of 1844 and 1852 (152, 153) had favored the appendicular theory. Huisgen (127), after investigating the ovary in a number of plant families, decided that when the ovary is inferior, its wall is formed by the hollowing out of the receptacle. He offered some original suggestions regarding the placenta, which, according to J. Reynolds-Green (106), made considerable headway at the time. Huisgen regarded the placenta as an axial structure in the Primulaceae, Lobeliaceae, Ericaceae; as an independent "blastem" in the Cruciferae and Resedaceae; and as a carpellary structure in the Violaceae, Leguminosae and Monocotyledonae. The dissepiments were considered to be outgrowths of the placentae meeting with the carpel edges—the view of Hofmeister. His idea of independent blastems may have come from Treviranus (245) who had suggested in his textbook that in certain cases the placentae might be independent structures of the nature of phyllomes. Hagen (116), who submitted his dissertation at Bonn the same year, explained the placentate, as well as the "false partitions" of *Mesembryanthemum,* as developments from growing points alternating with the carpels. He, however, considered that the ovary wall of this plant was made up of a calyx-tube fused to the ovary.

Barcianu (8), using the new Hanstein method in the preparation of his material, re-investigated the Onagraceae and came to the conclusion that the wall was axial but that the placenta was a new structure, developed from four primordia which arose from the axis in acropetal succession, in the same manner as sepals and petals; on this account he concluded that it was neither axial nor carpellary, but an organ *sui generis.* He noted that the ovules developed from swellings of the placental primordia, and he considered them as equivalents of leaf segments, whole leaves, axial sprouts or undifferentiated points. Dissepiments were formed by growth of the carpel margins. Reuther (189), influenced by the ideas of Huisgen, Hagen and Barcianu, later added the suggestion that these placental fundaments, arising from the axial cavity in acropetal succession, were the equivalents of the carpels, with which they alternated. It would seem as if the botanists of the time were vying with each other in a search for novelty—a sport which seems not to have been wholly confined to the eighteen-seventies.

This was too much for Čelakovský. Fearing that the old carpellary theory of de Candolle was becoming endangered, he turned to the investigation of the "cupula" (57). After making a comparative study of the hypanthium of a number of families, he reached the conclusion that neither the theory of the axial school, which he said was the prevailing one of that time, nor that of the appendicular, which was gaining ground, was correct. He saw in the hypanthium an axial cup which bore at its summit external floral verticels, concrescent with the external wall of a normal carpellary ovary (*Fig.* 4). He thought that it would seem very strange if the carpels of an inferior ovary were to remain in a rudimentary state, while they were fully developed in a superior one. Also, if the cup were to be regarded as entirely axial, it would be very difficult to explain the semi-inferior and inferior ovaries of the Ericaceae and Rosaceae. He believed that in all cases the ovules were borne on carpellary leaves. On the other hand, he thought that the view that the cupule was entirely appendicular was untenable from a morphological standpoint. His position was that of Naudin and Decaisne. He reported that Goebel and Strasburger held the same opinion.

Eichler (85, 86), who before 1872 had believed that the stem extended only to the base of the floral leaves, then had favored the view of Huisgen, now became a convert to this school of thought. In the light of the developmental evidence he regarded the vascular bundles to be "ein secundäres Moment", since the phyllome grows out from the inner surface of the floral cup.

In 1875 Van Tieghem's epic mémoire appeared in its expanded form (258), and in 1879 Van Tieghem made a further contribution in his paper on the rose (259). His application of comparative anatomy to the study of the flower showed in a spectacular way how the disposition of the vascular bundles in an ovary could furnish an excellent criterion for determining which structures were axial and which were appendicular. He noted that the bundles of the receptacular tube run up part way, oriented in a normal manner. At a certain point, however, the carpellary traces branch off, double back on themselves, and move inward to the carpels with the xylem and phloem positions reversed. He reasoned from this that the receptacular tube was axial up to this region but appendicular above, since the latter portion was comparable to the tube in other members of the family, in which appendicular bundles in all degrees of fusion could be found. This paper was read at a session of the Académie des Sciences in 1878. Duchartre was present and asked Van Tieghem how he would explain the structure of a proliferated rose, in which supernumerary carpels grew out above others below. Van Tieghem replied that the supernumerary ones in his opinion originated in a kind of fission ("dedoublement"), analogous to that which takes place in a compound leaf. To many others also floral chimaeras furnished convincing grounds for believing that the wall of an inferior or semi-inferior ovary was at least partly axial. Masters (171) stated that roses with leaves growing from their hypanthium surfaces showed conclusively that the "so-called calyx tube of these plants is merely a concave and inverted thalamus". Pear chimaeras were explained by him in the same way. Gravis (104) reasoned likewise regarding Duchartre's celebrated proliferated pear (illustrated in Goebel's "Organographie"). As late as 1915 Chodat (59) wrote that an abnormal fasciated quince furnished evidence for the belief that the wall of an inferior ovary was entirely axial.

Warming's important memoire, "De l'ovule" (267), which rejected the idea that the ovule was a bud and substituted the alternative that it was a new structure whose nucellus was the equivalent of a sporangium, knocked out from under the Schleiden theory its most important support—for obviously sporangia could be produced on fronds. Nevertheless, Trécul (244), who had himself in 1853 (243) demolished another by showing that leaves do not always develop in basipetal succession, still clung to his former opinion. He reasoned thus: since the placental bundles of an inferior ovary leave the stele before the parietals and become inverted (wonderful evidence for the appendicular theory!), they can belong neither to the "tallons" of the carpellary leaves, as Van Tieghem thought, nor to the axis. He said that they needed no further explanation than that they were placental bundles which found their significance in their connection with the sexual process.

In 1882 Emil Boutineau (29) made an extensive comparative study of the anatomy of the Rosaceae, and decided that throughout the family (excepting the tribe Pomeae, in which it was axial) the receptacular tube consisted of concrescent appendages. His failure to confirm the conclusions of Van Tieghem (259) and Bonnier (26) regarding the rose was due to a different interpretation, rather than a failure to note the recurrent bundles within the floral tube which he observed in *Calycanthus* also.

At the request of Sachs, Goebel in 1882 re-edited Sachs' fourth edition of his textbook, and published it under the title "Grundzüge der Systematik und specielle Pflanzenmorphologie." This is the familiar "Outlines of Classification and Special Morphology of Plants" (99) which we sometimes forget is basically the work of Sachs. Goebel stated in his introduction that in fulfilling his task he had considered it his business to make only such changes as seemed to be required by the literature that had appeared since 1873. The discussion of the ovary and the classification of types was left as Sachs had written it. When the topic of the ovule was reached, however, Goebel changed the presentation, since this structure could no longer be looked upon as a bud. In 1880 and 1881 Goebel's papers (96), which finally established the morphological identity of the sporangium throughout the vascular plants, had been running in the Botanische Zeitung, and settled for most botanists at least, the status of the much discussed ovule.

In 1886 Goebel (98) became sufficiently interested in the problem of epigyny to investigate it for himself. Up to this time he had seen little distinction between the appendicular and axial theories, because he considered that the appendices were not branches of the axis but excrescences of peripheral parts. After making an ontogenetic study of the bud of a pear, he decided that the inferior ovary represented the product of fusions and intercalary growth in which the whole of the vegetative point, including both axial and carpellary tissues, were involved. He noted that the carpellary anlage, instead of being confined to the edges of the cup, generally extended to its base, and that during development lengthening of the insertions of the inner appendicular organs kept pace with the growth of the axial zone on which they originated. He had always been impressed by the similarity between the superior and inferior ovaries, and held that comparative anatomy as well as ontogeny supported the view that they differed only in minor details. This position was taken in his "Organographie" (100), but he registered his impatience with attempts to determine the exact limits of the carpellary and axial structures. As to the insertion of the ovules, after calling attention to the situation in *Juniperus,* he said that he saw no reason why the change from carpel-borne to axis-borne ovules could not be brought about. "Everything else can change, so also can this. What we should endeavor to find out is the method and manner of *how* the change has taken place, and the *conditions* under which it is completed". The subdividing of the two ovarian types in this work was based upon whether the vegetative point of the flower was or was not used up during development. The following quotation is a familiar one (101):

"On account of deficient historical investigation, the view was formerly advanced that the ovary in the epigynous flower is formed from the cup-like flower-axis, and the carpellary leaves only produce the styles and stigmas. Comparative morphology has rightly contradicted this interpretation, which, however, is still found in many books. As the history of development shows, the carpels share in the construction of the ovarian cavity, and the ovules have no other origin than that which is found in the superior ovary. It is common in all inferior ovaries that the vegetative point becomes at an early period more or less concavely hollowed out, and that the leaf-structures of the flower sprout out partly from the margins, partly from the inner surface of the depression. Whether one describes the marginal part of the flower axis as a 'congenital concrescence' of the different leaf-whorls of the flower is an arbitrary matter, because the flower-axis ends its active existence with the bringing forth of the leaf-structures of the flower. The earlier the flower axis assumes the cup-like form, the more will we in general ascribe its character to the flower axis; the later this form is assumed, the more will its features approach the more primitive condition as we find it in hypogynous flowers. Where, as for example in many Cactaceae, the outer surface of the inferior ovary is able to produce leaves and lateral shoots, we can have no doubt about its axial nature; the flower-axis has here become drawn into the formation of the ovary at a late period. In other cases, however, this takes place very early, and then the axis appears, as has been said, to pass right back into the leaf-structures of the flower".

Strasburger (231) favored the hollow-axis-carpel theory, although we find him making the following comment: "It has, however, no other than a phylogenetic (or evolutionary) value;—in point of fact, however, the anatomical and physiological data of such a conception are wanting, and we must, therefore, be content with stating that the structure of the inferior ovary is not different from that of a polycarpellary unilocular superior ovary". Schimper and Karsten, in numerous editions of Strasburger's "Lehrbuch" (232, 234), as well as Pax (182) explained the inferior ovary on the basis of this theory. The latter, however, in commenting on the irreconcilability of the points of view of developmental morphology and comparative anatomy, added, "freilich wird immer zu untersuchen sein, ob nicht der unterständige Fruchtknoten durch Anwachsen der Blüthenhülle entständig ist".

Henslow is generally regarded as one of the followers of Van Tieghem, but he apparently believed that the concrescent appendicular ovary was enveloped on the outside by a very thin axial cup (123). After noting in the ivy that the respective cords of the calyx, corolla, stamens and carpel backs were fused together into common bundles, he remarked that he would not hesitate to call everything within an imaginary circle around them as foliar: "so that nothing would be axial except the boundary, *i.e.*, the epidermis and a certain amount of sub-jacent tissue". Farmer (91) had reported two years earlier that this plant furnished an example of a fruit whose pulp is mainly derived from the carpels. In 1888 Henslow (122) in the following statement had advocated the use of the term "receptacular tube":

"Hence it appears undesirable to call it either a calyx tube or axial; for these terms would seem to bind one to consider it permanently and in all cases as being either of one nature or the other. The term, 'receptacular tube', is therefore best, as it certainly 'receives' or supports the whorls of the flowers; and teratology clearly shows that it can be either foliar (petiolar) or axial, according to circumstances. . . . In the case of the inferior ovary, I would again emphasize the fact that the difficulty as to what is axial and what is carpellary is entirely removed, if the undifferentiated condition of the carpels be thoroughly understood".

By 1890 the hollow-axis-carpel theory had gone far on its way toward becoming the dominant theory. Schaefer (212), in an important article based on evidence from embryology, comparative anatomy and teratology, concluded that the axis functioned as a bearer of carpels in the inferior, just as in the superior ovary, the only difference being that in the former case the axis was concave, and in the latter, convex. Also, inasmuch as the view that the carpels were in a certain sense sporophylls was becoming adopted, the placentae would seem to be outgrowths from these in both types. Bertrand (17), who suggested that the inferior position of the ovary should be regarded as an advanced character, said: "En règele générale, l'infère-ovaire provoque une sorte de reçut pour la fermenture des carpelles".

Grélot (112), who carefully traced the course of the vascular bundles in representative genera of the Gamopetalae, was exceptional at the time in concluding that the semi-inferior ovary, when present in the group, developed out of fusions of the lower part of the perianth with the ovary wall. He noted that differences in strength or weakness of the bundles of the appendages are correlated with their functions; vegetative leaves tend to develop strong median veins, and weak or no marginals, whereas carpels are generally characterized by reduced medians and strengthened marginals—a development correlated with their role of supplying nourishment to the ovules. All these variations he attributed to the marvelous plasticity of the plant.

Vidal (264) in 1900 made a study of the summit of the axis in the Gamopetalae, and concluded that in syncarpous forms the axis might contribute to the formation of the pistil by either prolongation in the center as a placental column or by invagination and formation of the wall. He thought that the receptacular tube might be partly or wholly axial, depending on its height, and that the ovules might be derived from either the carpel margins or the axis. He claimed to have made use of evidence from vascular anatomy, in addition to those of ontogeny and teratology; but, as Kozo-Poljanski pointed out (146), he made no comparisons of the vascular systems of the flowers he used with those having comparable superior ovaries, neither did he take into consideration the bundle situation in the steles of the flowers he used.

The growing acceptance of the invaginated axis-carpel theory from 1875 until the end of the century was reflected in textbooks (*e.g.*, 5, 6, 73, 85, 86, 89, 182, 231, 232). Others, however, still adhered to the appendicular theory (13, 55, 144, 195, 260). On the other hand, there were a few who looked upon the true inferior ovary as an axial structure (*e.g.*, 31, 186, 197, 265).

Some botanists (*e.g.*, 18, 20, 77, 272) were not anxious to commit themselves. Bessey (18, 20), after a discussion of epigyny in his textbooks of 1880 and 1899, wrote: "Some cases of epigyny are doubtless to be regarded as due to the adnation of the corolla, stamens, and ovaries: in others the ovaries are adnate to the hollow axis which bears the perianth and stamens: in still others it seems probable that the hollow axis is itself ovule-bearing, and that the true carpels are borne on its summit". His retiring presidential address (19), given before the Botanical Society of America at the Toronto meeting in 1897, in which he stressed the great contribution which morphological studies rightly interpreted can make to phylogeny and taxonomy, contains the following paragraph:

"All apocarpia are free from the other organs of the flower, and this is the case with many syncarpia. There are, however, many syncarpia to which some or all of the outer leaves of the reproductive strobilus have become more or less completely attached. In the so-called epigynous flowers, as the irids and orchids among the monocotyledons, and the myrtles, cactuses, umbelworts, and all of the Inferae of the dicotyledons, there has been such a fusion of the originally separate parts of the strobilus as to result in a single compact structure, in which in extreme cases only the distal portions of the original leaves are distinguishable".

By 1914, however, Bessey (22) had come to favor the prevailing theory in regard to *Amaryllis*, "whose ovary is overgrown by the receptacular cup which carries the perianth and stamens", and for *Iris*. In his subdivisions of both monocotyledons and dicotyledons (21), he made use of hypogyny, which he considered primitive, and epigyny, which he considered derived. Flowers in which the axis was expanded into a disk or cup were called "cup flowers".

Asa Gray (105), also, who subscribed to the de Candollian theory in general, did not rule out the possibility that a hollowed axis might contain an inferior ovary in certain cases. He thought that the lower part of the pear represented the enlarged extremity of the flower stalk, as was true also of rose and cactus flowers, and summed up the matter in these words: "so it is most probable that in many cases the supposed calyx-tube adnate to the inferior ovary is partly or wholly a hollowed-out receptacle (in the manner of a fig-fruit): that is a cup-shaped or goblet-shaped development of the floral axis". When adnation occurs, he pointed out, the floral parts "are born united".

Many of the textbooks illustrated two types of epigyny, one in which the carpellary tissue lines the hollow receptacle, and another in which it is confined to the covering. The latter condition came to be considered by some as "true epigyny", as opposed to the "false epigyny" of the former. Rusby and Jelleffe (195), on the contrary, had used the term "true epigyny" in a still different sense, to specify adnation of all the floral whorls. These authors mention two kinds of "apparent epigyny", in which "the disk enlarges upward, surrounding the gynaecium and adhering to it", and "the end of the branch is hollowed, and the gynaecium sunken into and adnate with it; the other organs hence thus elevated above and apparently, but not really, upon the ovary or ovaries". "True epigyny", however, in the minds of most botanists of the early twentieth century came to represent an advanced type of inferior ovary, not very different from Schleiden's conception in structure, but having a different phylogenetic origin. In Clapham's refutation of Thompson's acarpic theory (61) we may find a clear statement of this concept. He wrote: "The inferior ovary is surely a derivative type in which the ancestral carpels have ceased to bear the ovules, and are represented only by the 'stylar components'. All stages between this and the hypogynous condition are known, and the essential change seems to be in the distribution of growth after initiation of the carpels on a concave receptacle. Growth of the carpels as free or concrescent members, independently of the receptacle, gives the superior ovary; but growth predominantly beneath the primordia, increasing the concavity of the receptacle or forming the loculi as pockets beneath the stem-apex, gives the inferior ovary. It is thus true that the ovary is not composed of carpels, but it cannot be maintained that it is ancestrally acarpous".

The less advanced type, represented by an invaginated axis, containing carpels, came to be considered as false epigyny or as extreme perigyny (194, 197, 265). Miss Thompson (238) recently placed the term "perigyny" in quotation marks, when referring to the basal adnation of floral whorls which she found in the Caryophyllaceae. This would seem to reflect a general opinion that true perigyny implies an invaginated axis. King (142) interpreted the perigyny of the olive as the result of retarded growth in the center of the torus, but other recent investigators (176, 150, 240) have clearly shown that the perigyny of the Leguminosae, Polygonaceae and Kalanchoideae is the result of adnation of the floral whorls. It is interesting to note also that Laubengayer found that the polygonaceous ovules, so long considered terminal and cauline, were in reality borne on very much reduced carpellary placentae.

Ganong's opinion regarding the epigynous flower is recorded in the following sentence (93): "The ovary of such a flower for example, unquestionably originated in sporophyllous leaves standing on a conical receptacle, precisely as in numerous flowers today; gradually, however, as embryology proves, the formation of the ovarian cavity was given up by the carpels, and assumed by the receptacle, which grew up in the form of a cup carrying the other parts upon its rim, while the carpels finally came to form simply a roof over the cavity". Ganong, however, was insisting that the inferior ovary was a new structure "which had acquired an identity and character of its own", during which process "the old characters of receptacle and carpel had melted away". On that account he thought that it would be useless "to expect that such an ovary would build placentae, partitions, styles, or stigmas, according to the rules in vogue with ordinary receptacle and carpel, and useless, also, to discuss whether in such an ovary the cavity is lined with carpel or not, for the ovarian wall is no longer either receptacle or carpel or both, it is ovarian wall; carpel and receptacle have not fused to form it, their tissues have melted away, so to speak, into the tissue which does form it".

Velenovský (261), however, was not satisfied that Van Tieghem was wrong, and turned his attention to flowers in numerous families characterized by jointed pedicels, such as *Asparagus, Hemerocallis* and *Hibiscus.* An anatomical and comparative study of these forms with others convinced him that the swelling below the constriction was morphologically different from the inferior ovary above. It consisted merely of expanded axis, whereas the hypanthium above represented a fusion of perianth, stamens and gynophore. He stated that he considered it unwarranted to regard the hypanthium of the Rosaceae as axial, in view of all the gradations of union between the hypanthium and carpels which exist throughout the family. He looked upon the inferior ovary of this family, as well as of the Saxifragaceae and Umbelliferae, as a concrescence of floral members. In his "Verleichende Morphologie der Pflanzen" (263) he said that he agreed with Van Tieghem in his opinion that undivided bundles furnished a criterion for recognition of an axis. Inasmuch as so much confusion existed regarding the use of terms, he suggested that the term "Becker" (cupule) be kept for the axial inferior ovary, such as is present in the oaks, and in the outer cup of *Eschscholtzia;* and that "Phyllome-Becker" (hypanthium) be used for this structure in other cases—the vast majority—which owed their origin to adnation of floral whorls.

Wernham (270) in 1911, after discussing the sunken ovary in the invaginated axis of the Inferae, commented as follows:

"It will be convenient to suggest at this juncture that inferiority of the ovary may, not improbably, have been produced in descent in more than the one way we have indicated, although this latter is alone in having left any continuous trace among existing plants. The point that we wish to make here is, that, whatever the evolutionary method of its production has been, the inferior portion of the ovary conduces to economy of production, since, in virtue of this position, receptacular tissue can be pressed into service of ovule production and of fruit formation. In any case it is certain that those floral types such as the Compositae, which are admittedly in the van of evolutionary development, invariably possess an inferior ovary, while no epigynous flower can be called unquestionably primitive".

During the early part of the twentieth century there was a comparative dearth of papers dealing with the inferior ovary; partly perhaps because most morphologists regarded the question as settled, and partly because they were much more interested in what went on inside the embryo sac. We have those of Kirkwood (143) who went back to the theory of Schleiden for an explanation of the inferior ovary of the Cucurbitaceae; also one by Hillman (125) on the Rosaceae, and three on the pome fruit by Kraus (147), Kraus and Ralston (148), and Black (24). As in the case of its behavior at our erstwhile Hallowe'en parties, the apple refused to stay down. Hillman concluded that the hypanthia of all genera of the family, with the exception of *Rosa,* whose hip was entirely axial, and the Pomeae, whose carpellary core was embedded in the peduncle, were appendicular. Kraus, Ralston and Miss Black decided also that the flesh of the pome was receptacular. (One, facetiously inclined, at this point might be tempted to ask if the flesh by any other name would taste as sweet.)

Work on the floral development of the dandelion interested Coulter in the ontogeny of other flowers, and as a result he reported that in every form studied, the first recognizable character to appear was that of the inferior or superior ovary (64). He concluded: "Now, if ontogeny means anything, plants with inferior ovaries must be regarded as more highly and recently developed than those whose flower parts are hypogynous". In "A Textbook of Botany"

(66) the general development of the flower is outlined as follows: The primordia of each floral whorl, after first appearing as separate growing points, tend to coalesce and grow up uniformly in zones. This zonal development is responsible for the so-called synsepalous, sympetalous, monadelphous and syncarpous conditions in different flowers. The tendency toward zonal development, furthermore, is not confined to single whorls but may involve two, three or four cycles. Where three sets grow up together, forming a tube around the carpels, the flower is perigynous; when four are concerned in the growth "en masse" the flower is epigynous. In regard to the origin of the ovules, Coulter thought that this might be either foliar or cauline, inasmuch as the tip of the axis sometimes is prolonged into the cavity, and the ovules may arise from any free surface. From this one might assume that he looked upon the wall as foliar, although in the "Morphology of Angiosperms" (67) Coulter and Chamberlain were careful not to ascribe either a carpellary or toral explanation to the floral ring which develops "en masse". The authors apparently agreed with Ganong in thinking that speculations concerning the morphological identity of ovarian structures were beside the point. Goebel's view as to the origin of some ovaries, however, is presented in the "Morphology" and followed by these words: "It is to be expected that numerous intermediate stages between complete hypogyny and extreme epigyny will be displayed, as may be inferred even from the doubtful phrases employed by taxonomists to describe them". In both "A Textbook of Botany" and "Morphology of Angiosperms" Ganong's illustrations of floral types are used.

Hannah (120) in 1916 summarized and reconciled the results of previous ontogenetic studies. She concluded that both monocotyledons and dicotyledons form their ovaries in the same manner, by zonal elongation of the tissue just below the point of origin of the floral leaves, the elongation taking place during or after appearance of the floral primordia.

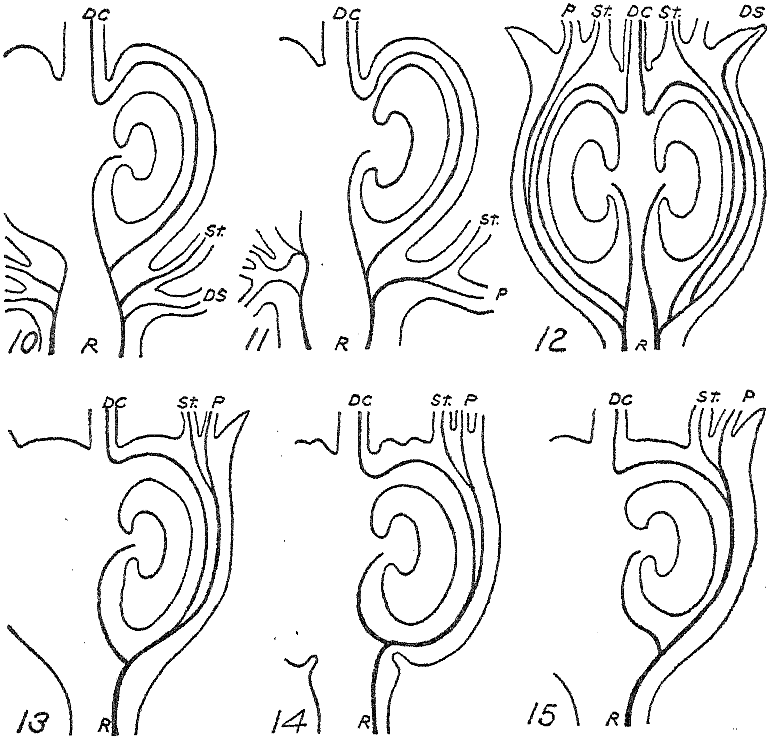
Of the textbook authors writing in the early twentieth century the large majority supported the hollow-axis-carpel theory (*e.g.*, 30, 45, 106, 141, 234, 268). Some considered it a structure *sui generis* (65, 66, 70, 94) and there were a few advocates of the appendicular theory (3, 27, 221, 263). Engler and Prantl (89) subscribed to the hollow-axis-carpel theory, although taxonomists in general continued to use the expression "calyx adherent to the ovary", following de Candolle, Bentham and Hooker, and Asa Gray. L. H. Bailey in his "Lessons with Plants" (4) changed his definition of epigyny to conform to the current opinion, and used it in this way in subsequent systematic works. Rydberg (196) gave as reasons for his axial interpretation of the tube of the Potentilleae the following: (*a*) petals and stamens cannot be borne on the sepals, because the petals are deciduous at the point of insertion on the margin of the tube and the joints at which abscission takes place should represent the bases of these organs; (*b*) the vascular bundles of the petals and stamens are not free through the tube as they should be if the sepals, petals and stamens were inserted at the base of the tube.

MODERN PERIOD

New interest in problems of floral morphology was aroused by the publication of Sinnott and Bailey's papers dealing with the phylogeny of angiosperms (225, 226), and from these dated a renewed appreciation of the value of anatomical research in the determination of plant relationships. Their concept of the primitive three-lobed carpel (*Fig. 7*), formulated after an extensive comparative study of angiosperm families, was a basic one which stimulated much research. Eames early became interested in floral anatomy, and many papers by his students show how valuable the contribution of vascular anatomy is to the solution of problems of plant structure and relationship.

One of the first of these investigations was made by Miss Hancy (119) on the Ericaceae. Her findings strongly suggested that forms which range from hypogyny through perigyny to epigyny would show transitional stages in the fusion of the bundles lying in the same radii when brought together in the floral tube. This turned out to be the case, as can be seen by the diagrams used by Eames and MacDaniels in their "Introduction to Plant Anatomy" (84). Later Eames (82, 83) amplified these studies and showed how adnation, which at first was confined to sepals and petals (*Andromeda, fig.* 11), had progressed through a stage in which the bundles were brought together within the floral tube (*Gaylussacia, fig.* 12) to one in which the bundles were completely fused up to the top of the hypanthium (*Vaccinium macrocarpon, fig.* 15).

Furthermore, he called attention to the fact that when the bundles do become free, "the separation is often a tangential split and not a radial division, as would be the case if those bundles were departing from the stele" (83). Significant stages from his more complete series are shown in (*figs.* 10–15). Unless one discards completely the evidence from comparative anatomy, it would be difficult to interpret the heath hypanthium on any other basis than that



FIGS. 10–15. Diagrams of ericaceous flowers to show stages in fusion of the vascular skeleton under adnation—after Eames (83): fig. 10, *Pyrola secunda,* a typical superior ovary type with no fusion of organs; fig. 11, *Andromeda glaucophylla,* with stamen base adnate to corolla, and the traces of these two organs fused nearly to the base of the stamen; fig. 12, *Gaylussacia frondosa,* the stamens, corolla and calyx adnate to the ovary, but the vascular supplies of these parts largely free; figs. 13–15, species of *Vaccinium,* showing progressively greater amounts of fusion of stamen and petal bundles and these with the dorsal carpel bundles—(13, *V. vacillans;* 14, *V. pennsylvanicum;* 15, *V. macrocarpon*). FT, DS, St., P, DC, VC, R indicate, respectively, the floral tube and the dorsal sepal, stamen, petal, dorsal carpel, ventral carpel and stelar bundles.

it is completely foliar. Eames had for a long time considered that inferior ovaries originated in this way oftener than was commonly thought. In addressing the morphological section of the botanical congress in Cambridge in 1930, he made the following statement (82):

"The inferior ovary represents adnation in its extreme form. Comparative studies, made with the understanding that fusion of organs ultimately brings about fusion of skeletal tissues also, demonstrate that the inferior ovary in nearly all—perhaps all—families has resulted from the adnation of the outer floral whorls to the carpels. Histological evidence of this is available, but abundant proof is supplied by the course of the bundles, and the manner of their forking or splitting".

Bechtel (12) decided that the seemingly simple ovaries of the Urticales had become so as the result of coalescence, adnation and reduction, during which the originally anatropous, laterally-attached ovule becomes basal and orthotropous by a sinking into the tissues. He made the suggestion, also, that all cauline ovules in angiosperms might have become so as the result of the same process—a suggestion supported by a constantly growing mass of evidence.

✓ In 1925 Saunders (206) applied her "leaf-skin" theory, proposed in 1922 (204), to the study of the inferior ovary, and reached a conclusion at variance with the view "generally accepted (without exception in the case of British and German writers)", namely, that it is a hollow receptacle with an appendicular lining. She reasoned as follows: "Since the shoot stem consists of an axial core, enveloped by a foliar covering (the leaf skin), in ovary walls where the bundles lie close to the outside (no more than two layers from it) and are used up in going to floral members, no part of the wall can be axial, and must, therefore, consist of concrescent appendicular members"✓. She admitted, however, that direct proof could be found only in forms with two layers of cells outside the bundle, but asked: "Since the concave-axis theory is disproved for one, why not for all?". In later papers (207–209) and in her textbook (210) she developed her ideas in accordance with her theory of carpel polymorphism, in which she proposed that two kinds of carpels, valve and consolidated, take part in the formation of angiospermous gynoecia. ✓(For a more complete review of this theory see 2, 7, 10, 83, 276). Her interpretation of the vascular anatomy of the numerous flowers that she studied led her to the surprising conclusion that perigynous flowers are fundamentally different from epigynous ("syngonous") ones (205, 206). In the former she thought that the axis did become concave and enclose the gynoecium. Both *Rosa* and *Pyrus* were considered perigynous in this sense. It is unfortunate that "she neglected the clear comparative evidence present in her own work", to quote from Parkin (180), for she studied a large number of flowers, carefully traced their bundles, and diagrammed them well. The "leaf-skin" is difficult to demonstrate, and all ovaries, excepting possibly those of the Cruciferae, can be better explained on the basis of the more simple assumption that the original carpellary unit was a 3–5-trace follicle, as others have suggested (60, 83, 136, 226, 253). (Many of Miss Saunders' conclusions were based upon teratological evidence. Thus far few investigators have accepted the theory of polymorphism.) Parkin (180) considered that it "raised difficulties greater than it could solve". Barton-Wright (10), in his excellent review of the theory and the controversy that it engendered, concludes: "Nevertheless, to the impartial observer, it does appear that carpel polymorphism has been applied a little too hastily in every direction in the interpretation of carpellary structures, and in many cases the evidence is of far too weak a nature to support an even *primâ facie* case".

Among the inferior-ovaried forms studied by Saunders was *Begonia* (206, 208, 209), the gynoecium of which she regarded as entirely foliar. The prominent wings were interpreted by her to be outward-turned, lateral extensions of three semi-solid carpels, whose dorsal bundles were located in the middle of each flat face, which was topped by a commisural stigma. This position was taken in contradiction to that of Bugnon (42) who, although he admitted that her explanation sounded logical, was forced to see "la nature exclusivement axile de la paroi ovarienne" (*Fig.* 6). He reached this conclusion after making a comparative study of the venation of the vegetative and carpellary appendages of the same plant. The top of the ovary appeared to him to be the base upon which the female floral leaves were inserted. The crescent-shaped bundles of the free parts of the styles were considered to be the homologues of petiolar traces, and the two laterals, which run down into the placenta, as stipular ones. He refuted Miss Saunders' contention that the large bundles of the flat faces were carpel dorsals, and showed that there is no need for considering the stigma to be commisural—a fact recently confirmed (118). In 1926 Bugnon decided that the cupule of *Eschscholtzia* was entirely axial (41), taking issue with Lignier (158) who regarded it as foliar and had explained the colarette as an outgrowth of the calyx, and Velenovský (263) whom we have seen decided that the outer cupule was axial and the hypanthium foliar. In 1928 Bugnon (43) declared that the anatomical basis for the theory of congenital concrescence was entirely hypothetical, for in every case "an axial wall, developed by an intercalary *accrescence*" seemed to him to be a more logical explanation of the facts than a "*concrescence* of organs lost to view".

In 1928 G. H. Smith (228) described the inferior ovary of the Calycanthaceae as a hollow cup, formed by the checking of growth of the apex of the carpellate floral meristem, with a consequent bulging upward of the peripheral part. Because of its peculiar vascular anatomy he considered that the depression represented a true invagination of the floral axis. The stele in cross section consisted of two concentric systems of vascular bundles, the inner one of which, connected with the outer by means of branches, was characterized by inverted xylem and phloem. This inner stele gave off branches to the ovules whereas the outer one supplied the perianth. As can be seen, the vascular pattern is similar to that of the rose, as had been noted (*Fig.* 18) (26, 29, 259). Smith interpreted the hypanthium as partly axial and partly carpellary, and suggested that inferior ovaries might have arisen in more than one way. It is interesting to note that Engler and Prantl (89) used *Calycanthus* as an illustration of an inferior ovary which consisted of a hollow axis lined with carpels. In 1928 Gabrielle Bonne (25) also reported results of an investigation of the rose, in which she made use of modern technique and compared it with other genera of the family. Both perigyny and epigyny appeared to her to result from a congenital union of the receptacular tube—which she suggested be renamed the floral tube ("coupe floral")—with the carpels in all genera of the family excepting *Rosa,* whose hypanthium showed the invaginated bundles first noted by Van Tieghem, and was in consequence partly axial and partly carpellary.

A very interesting type of semi-inferior ovary, occurring in the Australian species, *Doryanthes excelsa,* was described by Newman

(178) in 1928. He noted that in the developing fruit "cohesion between the carpels failed to occur, while meristematic activity joined the carpels and the perianth-stamen tube together". The result was a flower which he could not call "really epigynous". It was rather "like a perigynous flower in which each carpel is adnate to the tube". A similar adherence between carpels and calyx-tube, but which formed a structure which was slightly sunken in the receptacle, was noted in the genus *Minuartia* of the Caryophyllaceae (172). Newman noted also that the *Doryanthes* flower is unusual in another respect. The ovules are very definitely produced on the abaxial side of the inrolled leaf, which he interpreted as excellent proof of the foliar nature of the carpel, and in line with Jeffrey's inclusion of the Angiospermae in the Pteropsida. Other amaryllidaceous species have been studied recently (113, 133, 134). Making use of the evidence derived from both ontogeny and vascular anatomy, Grove decided that the hypanthium of *Agave lechuguilla* represented a conjoint development of floral members. Joshi and Pantulu reached a similar conclusion regarding *Polianthes tuberosa.* Although White (271) did not focus his attention on the nature of the inferior ovary in his ontogenetic study of the flower of *Musa,* an ovary consisting of a tepal whorl adnate to the three carpels may be inferred from his diagrams. Juliano and Alcala found this to be true of *Musa errans* (137).

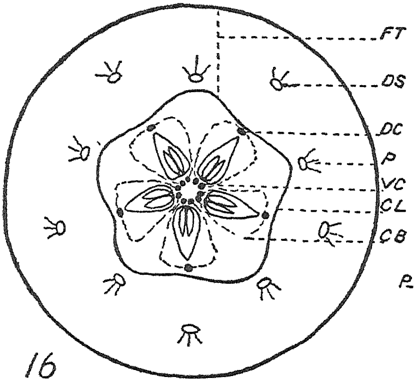
The year 1928 saw the birth of a new school of thought in regard to the morphology of the flower, to which Mrs. Arber has given the name "Gestalt morphology". It originated with Troll who revived the classical or idealistic, as opposed to the phylogenetic, approach to the study of the flower, by advocating comparisons of all flowers with a basic type. An excellent review of Troll's ideas by Just has been previously published in this journal (276). Briefly, Troll (250, 251) considers that the carpellary unit is a peltate structure similar to the aciculate leaf of *Darlingtonia,* which during development manifests its peltateness in varying degrees. The majority of forms have pitcher-shaped primordia; the minority, horse-shoe-shaped ones. These carpels build up a typical gynoecium which is syncarpous (plurilocular with central placentation) at the base, paracarpic (unilocular with parietal, basal or central placentation) in the middle, and apocarpic (with several free carpels) at the top. From this general type all others are derived by increases or reductions of growth within the three different regions (248–249). In regard to the inferior ovary, Troll (249) took the position that the floral axis could become a part of the gynoecium by penetrating its center, or by forming a cup on the outside. Witmer (277) interpreted the ovary of *Vallisneria spiralis* as an apocarpous one in which axial tissue could be found in the outer wall and between the carpels.

Leinfellner (151), another follower of Troll, made an ontogenetic and histogenetic study of the inferior ovary in *Selenicereus Macdonaldiae, Pereskia bahiensis, Eryngium planum, Pitcairnia xanthocalyx, Saxifraga Aizoon, Gladiolus segetum, Canna indica, Manettia inflata* and *Coprosma lucida.* He concluded on anatomical grounds that the carpels are peltate structures, enclosed in a hollow axis, with which they are congenitally fused at the cross zone. Since his observations revealed that the outer wall of the ovary originated in more deeply embedded cells of the *corpus* than the tips of the carpels and other appendages, he concluded that it was axial. The appendicular structures appeared to arise from the third layer inside. Whether histogenetic research is capable of furnishing definite criteria for recognition of axial and carpellary structures is for the future to decide. The small amount of evidence available at present is conflicting. McCoy (166) noted that all the floral organs of *Frasera caroliniensis* arise in a manner similar to that of the leaves of a vegetative shoot, by periclinal splittings of the tissue of the inner *tunica.* Brooks (35), on the other hand, reported that the floral apex of *Amygdalus communis* differs from the vegetative in the possession of but one tunica layer, instead of four, in consequence of which the whole floral apex is involved in production of the carpel. Satina and Blakeslee (203), by means of induced polyploidy, have been able to demonstrate three germ layers in the shoot apex of *Datura.* They note that the various components of the carpel originate in the innermost meristematic layer (L III), from which they infer that the carpel of this form is of axial origin.

Some other histological data exist which are of value as contributory evidence. Both Van Tieghem (258) and Henslow (122) called attention to the yellow band of parenchyma cells in *Alstroemeria versicolor,* which marks the junction of the receptacular tube, containing separate bundles of sepals, petals and stamens, and the ovary (*Fig.* 9). Bonne (25) noted the homology of the peripheral system of supernumerary bundles of *Lindleya,* a rosaceous form with a free ovary, with the surface system of the Pomeae. She also cited Murbeck's conclusion that the prickles of the Nuradeae were the equivalents of the bristles of *Agrimonia.* Occasionally *Epilobium* flowers are developed with semi-inferior or superior ovaries. Velenovský (263) noted that the hairs which cover the outside of these types are exactly like those which clothe the inferior ovary of the normal type; they led him to the conclusion that the outside tissues of all three forms are morphologically identical. The presence of septal glands, bounded by epidermal layers, which alternate with the locules in liliaceous flowers is well known and generally conceded to indicate regions where the fusion of the carpels is incomplete. Stomata in the endocarp of ericaceous fruits, noted by Bergman (15), would be difficult to explain on any basis other than that the endocarp is of epidermal origin.

Judson (135), influenced by the work of Kraus on the apple, took up the study of the Cucurbitaceae. He decided that the ovary wall must be receptacular, since it was formed by zonal growth of the meristem at its base. Inside the depression he noted three carpel primordia arising from the wall and developing as in a superior ovary, excepting that the margins on reaching the center were reflexed again toward the outside. Earlier papers (189, 143) had treated the placentation as axial.

In 1933 Jackson (130) published the results of a study of the carpophore of the Umbelliferae, which structure, "the core" of the ovary, she found to be appendicular, except for a small basal axial portion, present in most forms. She noted that the mericarps were somewhat unusual in that their ventral carpellary traces were modified into a central supporting structure, while the work of supplying the ovules had been shifted to the median-laterals. She decided that the disk and stylopodium represented expanded bases of the style. Some (51, 247) had considered this ovary to be entirely appendicular, whereas others (11, 28), on grounds of ontogeny, have looked upon it as axial. Hyde (129) in 1933 made an unusual suggestion regarding the gynoecium of the Campanuloideae in a paper read before the British Association at Leicester. According to Bancroft (7), he thought that it was composed of a whorl of similar carpels, each of which enclosed a loculus and a bifid "ovuliphore". The latter structures owed their origin to the floral axis and the carpellary axil.



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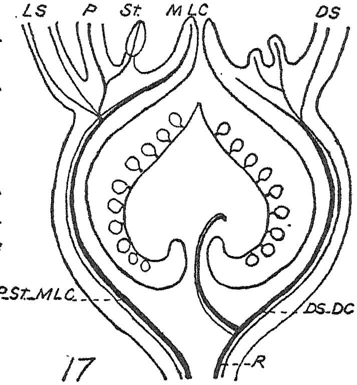

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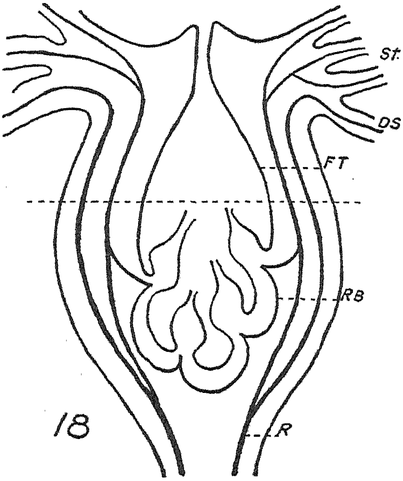

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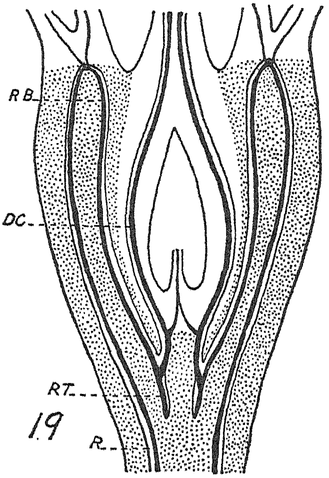

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FIG. 16. Cross section of an apple fruit—after MacDaniels (164). The floral tube (FT) is considered to be appendicular, containing sepal (DS) and petal (P) bundles. Within the core line (CL) dorsal bundles of the five-carpelled ovary (DC) connect by branches (CB) with their respective ventral bundles (VC). Many regard the flesh as receptacular. FIG. 17. Longitudinal section of *Samolus floribundus*—after Douglas (75). Within the ovary wall bundles lying in the same radii, the dorsal sepal-dorsal carpel (DS-DC) and petal-stamen-median carpel traces (P-St.-MC), are united up to the top of the ovary, where they become "free" by tangential splittings. FIG. 18. Floral cup of *Rosa*—after Jackson (131). Recurrent bundles (RB) indicate that the ovary is axial at the base (approximately below the dotted line) and appendicular above. FIG. 19. Longitudinal diagram of *Darbya*—after Smith and Smith (227). That epigyny is the result of a truly invaginated axis containing a carpellary ovary is indicated by the recurrent bundles (RB) and the residual tissue (RT). Stippled areas represent receptacular tissue.

Jackson (131) had been making a comparative study of *Rosa* and related forms when Bonne's paper appeared in 1928. Believing that the latter important contribution would not have a wide circulation because it had been privately printed in France, she published in 1934 the joint results and conclusions of her work and that of Bonne, which differed only in minor details (*Fig.* 18). MacDaniels (164) also, especially interested in the Rosaceae because of its inclusion of pome fruits, was not willing to accept as final the opinions of Kraus, Ralston and Black, and in consequence made another comparative study of the family with special reference to the apple, pear and quince. His conclusions, based upon an unbiased weighing of the evidence, leaves little room for doubt that the flesh is appendicular rather than receptacular (*Fig.* 16). (Textbook writers please note!) MacDaniels pointed out that there was practical value connected with the determination of the correct nature of the parts of the fruit, since they react differently to storage conditions. Mac Arthur and Wetmore (163) found that the carpellary tissues of the Wagener and MacIntosch varieties of apples could be differentiated from the outer tissues early in the development of the bud by staining reactions. On the basis of anatomical evidence, they were inclined to regard the flesh as floral. The apple problem, however, seems by no means settled, for Tukey and Young (252), believing that ontogenetic considerations outweigh phylogenetic ones, have recently supported the Kraus and Black interpretation. Yen (280), working on the fruit of *Ribes aureum*, noted that the vascular bundles of the wall were arranged in a single ring, which he thought made impossible their interpretation as either toral or carpellary.

Lindsey (160), in presenting evidence for full family status of the Menyanthaceae, called attention to the extreme degree of cohesion and adnation of the vascular traces of the outer whorls, which was reflected on the outside by a development of rind—a character lacking in the Gentianaceae. Within the latter non-epigynous family, the tribe Erythraeinae is characterized by a corolla-stamen tube that, although carrying its own bundles, is so thin that it secures its mechanical support by closely investing the ovary wall.

In 1938 W. H. Brown (38) called attention to the correlation which exists between development of the nectary and primitiveness or advancement of the flower. The lower monocotyledons, possessing septal glands or openings near the base of the flower, are hypogynous, whereas the higher monocotyledons, with glandular openings near the top of the hypanthium in a position which would allow for fusions to take place between the calyx or torus and the ovary wall, are epigynous. In dicotyledons development of a disk at the base of the ovary is characteristic of the lower forms, while a nectary ring, arising between the stamens and carpels and growing into a cup which partially or completely covers the ovary, is correlated with perigyny or epigyny in the higher groups. He suggested that the disk of the intermediate Ericaceae, originally located at the base of the ovary, might have become detached during the course of evolution and carried to the top by the upward growth of the torus. Gunderson (114), after making measurements of the depths of ovaries in buds and mature flowers of many species, said: "It appears to be a general fact that in buds of epigynous flowers the ovary is more nearly superior than in adult forms. Epigyny is recognized as being more advanced than hypogyny. The greater protection of the ovules must be considered an advantage to their development".

The present taxonomic opinion concerning the unnaturalness of the Amentiferae is substantiated by the evidence from vascular anatomy. Working on the Betulaceae, Abbe (1) noted adnation between the bundles of the sepals and the ovary wall in *Alnus,* and proposed from a comparative study of the very much reduced flowers which occasionally gave a clue, "that the synthetic ancestral form was a hemaphroditic flower with a (probably sympetalous) hexamerous perigone united to a tricarpellary gynoecium". The Fagaceae has been recently investigated by Berridge (16) and Reece (188). Both confirmed the opinion of Prantl (89) that the cupule represents a structure which has been evolved by the fusion of dichasial branches whose florets have been lost in the process of reduction of the inflorescence. Miss Berridge concluded that the inferior ovary had resulted from the coalescence of the ovary and the receptacular cup, which "would have tended to preserve the vascular supply of the carpel wall from complete disappearance, since it becomes merged in the general supply of the whole flower". Reece, however, in an excellent thesis which certainly should be published, took issue with Berridge in her interpretation of the structure of the inferior ovary wall, since he found "no anatomical evidence for interpreting the outer layer as an invaginated receptacle: it showed rather the nervation of a floral envelope".

Casimir de Candolle (54) explained the ovary of the Juglandaceae as a coalescence of one or two perigones, made up in various ways of sepals, bracts and bractioles, to an ovary which contained an elongated placenta bearing a single orthotropous ovule. Van Tieghem (255), in calling attention to this adhesion in *Juglans regia,* said that the ovary was erroneously called inferior, because the vascular systems of the appendages were separate in the floral tube. In 1909 Benson and Welsford reinvestigated *Juglans regia* (14), confirmed Van Tieghem's observations and illustrated their account with a series of diagrams which Van Tieghem's lacked. They stated that they found "no trace of that form of epigyny which is brought about by a concavity of the axis". Eames' diagram (*Fig.* 126, "Plant Anatomy") illustrates a concrescent floral tube enclosing bundles of each separate whorl. Manning (169) noted progressive degrees of adnation between the bract or bractioles and the pedicel and ovary, and also a fusion of the sepals with the ovary, when they were present. Others (149, 179, 223), on the contrary, considered that the ovary was largely axial. Shuhart found in the developing bud of *Hicoria pecan* three concentric rings of vascular tissue, the central one of which was characterized by inverted xylem and phloem, which led him to believe that he was dealing with a truly invaginated receptacle which enclosed an axial placenta. He attributed the splitting of the receptacular husk into four valves to a separation along parenchyma rays. He suggested that the fruit of *Juglans* could be interpreted in the same way, if one were to consider the middle stele, of which he found a trace, to be much reduced. If he is correct in his observations, these fruits would be of the same type as those of *Calycanthus, Rosa* and *Darbya.* Landon agreed with Shuhart in his axial interpretation of the urnlike tube and placenta, but thought that the septa were in part carpellary. One may gather that the evidence from this family is not all in.

The same may be said for the Compositae. In 1818 Robert Brown (36) called attention to the fact that de Candolle's character, "ovarum inferum", applied to the Dipsacaceae, did not always hold, since in some species the union between the ovary and calyx took place only near the narrow apex of the tube, while the parts below were free, thus producing the unexpected combination of "flos superum" with "ovarium librum", which he thought shed light on the place of origin of the floral members. Koehne (145), as we have noted, adopted the appendicular theory. Others (40, 69, 115) decided from ontogenetic evidence that the ovary was axial. Haenlein, however, agreed with Koehne in regarding the disk as a swelling of the base of the style, whereas Buchenau considered it axial. Both Cramer and Haenlein looked upon the ovule as a leafy organ. Coulter (63), after studying the development of the dandelion flower in 1883, decided that the view that the primitive ring was axial was untenable for two reasons: the appearance of the calyx is late, and the corolla lobes appear with the ring, not after it, indicating that the ring belongs to the floral organs. He thought, however, that the ovules were axial-borne, and that the carpels formed the arching roof. Baillon (6) described the gynoecium of the Compositae as an axial cup containing an ovary. Martin (170), after studying the early development of flowers in *Aster* and *Solidago,* noted that in the tubular ring which grew up from the periphery of the floral axis there was complete fusion of the floral members until liberated. After saying that there were "some who would substitute the word *hypogynous* for *epigynous,* basing their argument on the theory that all floral organs in their initial stages are coalesced in the annular wall", added: "The real origin and behavior of the floral organs in their younger stages of development as correlated with the inferior ovary has attracted but little attention and therefore no definite statement can be made as to the true relationship existing between the floral organs in their embryological development". Szabó (235) concluded that the inferior ovary of the Compositae represents an axial cupule but that the disk is a rudimentary structure, originally composed of four carpels, three of which have degenerated. Stebbins (230), in a recent monograph of the Cichorieae, called attention to the lack of adnation between the perianth and ovary in *Dubyaea* and suggested that this tribe is not far removed from forms characterized by superior ovaries.

Since the resurgence of studies based upon comparative anatomy the majority of modern textbooks have continued to support the hollow-axis-carpel theory. A few of the later ones, however, have adopted the theory of concrescence of floral whorls (*e.g.,* 72, 124, 224, 229, 241, 269, 275). Robbins and Rickett (193) make the following comment in a footnote (p. 500): "It is always difficult to say how much of the basal part of a flower is torus and how much is composed of the basal portions of stamens, petals and sepals united together. For class treatment it is simpler to consider the portion which supports or surrounds the pistil the torus, even though it may be shown that portions of this structure, or even all of it, are composed of leaves". Pool (185) thinks that the inferior ovary is not necessarily exclusively receptacular in every case. Schaffner (214), who supports the hollow-axis-carpel theory, divides epigynous flowers into three types: (*a*) without a hypanthium, ex. *Vallisneria;* (*b*) with a tubular hypanthium, exs. *Opuntia, Helianthus, Amaryllis;* (*c*) with a solid epigynous hypanthium in which the style represents a continuation of the lower part of the hypanthium, exs. *Iris, Strelitzia.*

Confirmation of the view that when there is an unquestioned invagination of the receptacle and a sinking of the gynoecium within it, as in *Rosa,* and in *Calycanthus,* the vascular anatomy tells the story, has come from some recent work by Smith and Smith (227) on the Santalaceae, a family which has long been a puzzle to morphologists. These investigators noted in the cupule of the flower of *Darbya* (*Fig.* 19) the same recurrent bundles that have been found in *Rosa* (25, 131, 259), *Calycanthus* (228) and *Hicoria* (223). They conclude that the outer tissues which surround the ovary and contain the recurrent bundles are axial, but that the placental stalk which contains the typical ventral bundles of the four carpels is floral. They note further that the free central placenta of some of the genera is formed as in the Primulaceae by reduction of the dissepiments. Additional evidence for their conclusion that the outer wall represents a true invaginated receptacle comes from their discovery that the residual tissue (vascular portions of the stele which may remain after all traces to floral parts have been given off (*Fig.* 19, R.T.)), is directed downward rather than upward, as is usual when it is present. In 1937 Schaeppi and Steindl (213) had suggested also that the outer wall of the *Osyris alba* ovary consisted of receptacular tissue. Following the interpretation of Troll, however, they had described the gynoecium as a paracarpous structure, consisting of peltate carpels, the cross zone of which is given over to the development of a carpellary central placenta, containing reduced central strands, which possibly represented an axial core. Earlier Dowding (76), an adherent of the theory of carpel polymorphism, had concluded that the ovary was entirely appendicular and consisted of an outer wall composed of five sterile carpels and a placenta made up of five fertile ones. Smith and Smith concluded that the anatomical evidence which is now available from the Santalaceae and other families "indicates that there are at fewest two types of inferior ovaries. One type, the result of extreme adnation, is entirely floral in nature. The other type, described here, would seem to have resulted from invagination of the floral axis followed by fusion to the ovary of the receptacular portion of the resultant cup".

It would seem, then, that for those who still cling to the classical theory of the foliar nature of the carpel, two types of inferior ovaries, differing in their method of origin, may be recognized; but that, contrary to the general opinion of the last century, the one which has arisen by adnation of the floral whorls rather than by an invagination of the axis, is by far the most common. At the present state of our knowledge the possibility that there is a third type, in which the ovary is axial and the carpels represented only by styles and stigmas, should perhaps not be discarded. If, however, such does exist, in all probability it has been derived by extreme reduction from one of the above-mentioned types. The evidence is increasing in support of the view, held by Cĕlakovský, Warming, Alexander Braun, Van Tieghem, and others, that the ovule of the angiosperms is always foliar in origin. When the "terminal ovule" of the Urticales and Polygonaceae turns out to be appendicular (12, 150), we become suspicious of all. The type of ovary characteristic of *Najas* (44, 165), with its seemingly axis-borne ovule, is a case in point. This type of much reduced ovary, however, would not be comparable in phylogenetic origin, at least, to the one visualized by Schleiden, although it would be similar in structure.

Unfortunately, however, the well tested classic theory had been under attack from several quarters, and we cannot dispose of the problem so simply. We have already mentioned the polymorphism theory of Saunders and the Gestalt theory of Troll. Thomas (236, 237) thinks that "the theory of the rolled leaf is too naïve", and that the classical morphology, based upon ideas of Goethe and de Candolle in which evolution had no place, should give way to the "new morphology" in which the ascent of the Angiospermae from the Psilophytales through the Pteridospermae should be given consideration. According to Thomas (237), "The whole carpel is deemed to have originated from a branched structure by evolutionary stages totally different from those passed by the foliage leaf, though both structures are regarded as originally derived from specialized branches of the thallus. The ovules are held to represent original terminal structures, the placentae separate branches, and the wall a cupular structure quite different in origin from a typical foliar structure". Structures which are very suggestive of such primitive carpels are to be found in the Caytoniales. The fossil evidence, derived from many sources, suggests that "the hollow cup-like receptacle may be quite as primitive as the conical receptacle of hypogynous flowers".

J. McLean Thompson (239) holds that it is futile to look for clues in past history other than the existence of a sporogenous axis, and bases his "developmental morphology" on ontogeny alone, believing that "the flowers will be their own interpreters, if their full development is known". Briefly, his theory is this: the flower itself is a sporogenous axis, save in its basal portion, which represents a transition from the vegetative body to the spore-bearing terminus. Bracts, bractioles and sepals are of the nature of transitional foliage; the lower portion of the sporogenous tissue represents microsporangium, and the upper or inner part, megasporangium. Emergences arising from the lower part of the axis raise up the potential microsporangium, thus producing stamens and, when sterilized, staminoidia and petals. The final position of all the members is determined according to the relation between apical and toral growth, the latter usually being dominant over the former, thus bringing about a cup-shaped axis. Toral growth carries up the emergences, including some that are diverted from spore-bearing into glands and stigmatic surfaces. The surface of the axial cup is potential megasporangium. Emergences from the axis produce the placentae which in time develop emergences, ovules which bear the nuclei (portions of megasporangium tissue). The inferior ovary is the result of dominance of toral growth over axial growth, and the superior ovary of axial over toral. Neither type is derived from the other, since both express states of flowering with a common basis. He believes that the cause of stigmation, fertility and secretion is a matter for physiological study and that the vascular anatomy is significant in relation to the nutritive needs of the floral organs rather than to phylogeny.

A theory similar to that of Schleiden in modern dress has recently been advanced by Hagerup (117). He regards the ovules as miniature branches but of the nature of monosporangiate macrosporophylls. He considers the placenta to be a prolongation of the axis which functions as a bearer of these macrosporophylls, and the carpels as involucres of coalesced and sterile leaves. In line with this reasoning he looks upon the inferior ovary of the Cactaceae and Aizoaceae as a hollow axis producing parietal reduced macrosporophylls inside and sterile bracts outside. Unruh (253), who regards Hagerup's idea of the ovule to be but a revival of the 70-year-old one of Cramer, notes that Hagerup's conclusions are not based upon the structure of the ovule but on the distinction between appendicular and axial organs—"the hundred-year-old obstacle".

Still another theory needs to be considered. Grégoire (109–111) has proposed that the flower is a structure "sui generis." He denies categorically that it is the homologue of the vegetative shoot, while insisting that the theory of metamorphosis is not applicable to the flower. He bases his denial on the fact that sequence in growth of floral leaves is away from the axis, rather than toward it, as in true leaves, and that the veins of the former develop in an acropetal manner, whereas those of the latter are basipetal; also floral primordia are without buds; and finally, floral organs do not compare with sporophylls, neither is the ovule a megasporangium, nor the embryo-sac a megaspore—quite a list of negations of ideas rather generally held! He admits, however, that the stamens might be comparable to microsporangiophores, and the carpels to spermatophores. In other words, he looks upon the flower as a meristem-carrier which ceases to grow after its mission has been fulfilled. In line with this reasoning he denies that the inferior ovary is a product resulting from adnation of floral parts, or of collaboration between axis and gynoecium, in concluding that the flower represents only the growth of a transitory structure implanted on a permanent leafy body. Miss Esau (90), however, after having made an exhaustive review of the literature concerned with primary vascular tissues in seed plants, recently made the following statement in this journal: "It has been sufficiently emphasized in this review that the universality of basipetal differentiation of traces in vegetative shoots, as assumed by Grégoire, is under serious doubt. References to acropetal development of procambium in vegetative apices appear to be on the increase in morphological papers. If the continuous acropetal differentiation should prove to be a common characteristic of the vascular meristem of shoots, one of the fundamental differences between vegetative and floral apices, as conceived by Grégoire, will be broken down". The most devastating comment upon Grégoire's doctrine was made by Goebel (102) when he wrote that one could be a splendid cytologist or anatomist and at the same time an utter stranger to morphology!

Space does not permit following further the controversy which has resulted from the emergence of these various theories. The old carpellary theory, which has been so ably defended (*e.g.*, 2, 146, 181), may need a somewhat different interpretation, but as Scott (222) has said: "will probably continue to hold the field until more definite evidence can be brought against it". Mrs. Arber, with her usual lucid gift of expression, wrote: "Considering the range of variation among gynaeca, it was a bold measure on the part of early morphologists to seek to bring them all under this simple and uniform conception, but the event has proved that it 'works'. Engler (1926), for instance, after more than fifty years of taxonomic work, stated definitely that he adopted the foliar carpel view for all the angiosperms". Again we quote from Parkin (181): "In conclusion one may say that though these attempts to reach a new conception of the Carpel are welcome, none of them is likely to be accepted whole-heartedly. The time has not arrived when we can abandon with equanimity the classical interpretation. There is nothing at present very satisfying to put in its place".

SUMMARY

Since the time of Linnaeus many theories have been advanced to explain epigyny in plants. Most investigators have accepted the concept that the flower is the morphological homologue of a short shoot, consisting of a determinate stem bearing a collection of leaves or sporophylls, the carpel being regarded as the equivalent of a folded leaf or sporophyll.

The following explanations have been offered to account for the inferiority of the ovary by those who have accepted the "classic

theory" of the nature of the carpel. It represents: (*a*) a concrescence of the bases of the calyx, corolla, androecium and gynoecium, and is thus appendicular (School of de Candolle and Van Tieghem); (*b*) a hollow receptacle, modified for reproduction, and is thus axial excepting for its carpellary roof (School of Schleiden); (*c*) a hollow receptacle lined and concrescent with a carpellary core (School of Naudin, Čelakovský, Goebel); (*d*) an axial wall, containing a carpellary placenta (School of Sachs and Bugnon). In (*a*) and (*b*) the axis may penetrate the center as a carpel bearer.

Other theories have postulated a different origin of the carpel. Troll and his "Gestalt" school consider that it is a modification of a peltate leaf of the type found in *Darlingtonia*. H. H. Thomas holds that the primitive carpel was similar to the cupule characteristic of the Caytoniales ("The new morphology"). J. M. Thompson, on the other hand, thinks that it is futile to look for clues to past history, and visualizes the flower as a sporogenous axis in which apical and toral growth are interacting, the inferior ovary resulting from a dominance of toral over apical growth. Hagerup considers it a hollow axis which produces on the inside reduced monosporangiate macrosporophylls, and Grégoire, a structure *sui generis*—a meristem-carrier which ceases to grow after its mission has been fulfilled.

The majority of botanists, however, have been reluctant to abandon the classical theory, since the foliar carpel view seems to be the best explanation to account for all the facts which have been accumulated in the course of the many investigations of the flower by the use of all methods—those of descriptive morphology, comparative morphology, ontogeny, histology, teratology, comparative anatomy, vascular anatomy and paleontology. Until recent times, conclusions have been based on the results obtained by the employment of one or two of these methods, with the result that they have been inconclusive. This is especially true of studies based upon teratology or ontogeny. Recently we have witnessed a re-emphasis on the method of comparative vascular anatomy which has furnished criteria for determining when the outer wall of an inferior ovary consists of a truly invaginated axis, and when it represents an adnation of floral appendages. In the former case, the stelar bundles, running to the top of the ovary, after giving off traces to the three outer whorls, bend backward, and in the course of their downward descent give off the carpel traces. In the latter case there exist all degrees of fusion between the bundles of adnate floral whorls before the tangential splitting takes place which frees them from each other, but there is no invagination. Thus two types of inferior or semi-inferior ovaries can be demonstrated. Contrary to popular opinion, however, the type which is characterized by a truly invaginated axis is rare. Thus far we know it only in *Rosa, Calycanthus,* the family Santalaceae, and possibly in the Juglandaceae. In the majority of other flowers the inferior ovary appears to be a structure composed of the fused basal portions of the calyx, corolla, stamens and petals adnate to the fused carpels.

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THE CLASSIFICATION OF INFLORESCENCES

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INTRODUCTION

To a science which prides itself upon precision of language, any confusion in terms is a reproach. The terms of systematic botany enjoy a respectable antiquity, but sometimes lend a regrettable obscurity to the scene. The names applied to inflorescences were confused from the beginning. In current manuals they are usually classified as determinate (cymose) or indeterminate (racemose). Umbels and panicles are included among the latter; yet the same manuals will refer to the compound determinate clusters of *Allium, Pelargonium* and *Asclepias* as umbels and to the aggregated cymose clusters of *Gilia* and *Penstemon* as panicles. The inflorescence of *Claytonia* is commonly described as a raceme, whereas to a more than casual glance it is evidently cymose.

Usage of botanical terms can not be settled, like that of botanical names, by a principle of priority; it would not advantage the science to compel taxonomists to adhere to the definitions of Linnaeus. An alternative is to adopt current usage; in view of the somewhat chaotic conditions indicated above, this also seems an unreliable guide. To reverse the old dictum: Res si nescis, perit et cognitio nominum. A rational terminology mirrors that upon which it is based—an understanding of the things concerned. To discuss the terms which have been applied to inflorescences (or to flowers, or to fruits) is to examine the concepts through which our current understanding (or lack of understanding) of these parts has been developed. Such a survey may indicate the problems that await investigation, besides clearing away the accumulated rubbish of the ages. In short, an attempt to arrive at a rational terminology is *ipso facto* an effort to evaluate scientific concepts and generalizations.

Much knowledge of inflorescences has accumulated since the first great flowering of botany in the eighteenth century; and many theories. Unfortunately, names and classes of inflorescences have been proposed under the influence of certain theories now discredited or which ought to be discredited, and in the absence of facts now available; the names have outlived the theories, to our ultimate confusion. This situation is not peculiar to the study of inflorescences; it is my contention that an historical study of terminology may illuminate many of the more murky corners of descriptive botany. It is with this in mind that I offer an essay on the classification of inflorescences.

DESCRIPTIVE TERMS OF THE EIGHTEENTH CENTURY

For convenience we may regard Linnaeus' "Philosophia botanica" of 1751 (69) as the beginning of the terminology of taxonomic botany. The terms, of course, were not original with Linnaeus. Since it was natural for a scholar of the eighteenth century to write in Latin, he used Latin nouns and adjectives as descriptive terms, without intending a specialized technical vocabulary unintelligible to all save botanists. The words had been in use—often in various senses—since the time of Pliny and before. But the great systematist was among the first to assign fixed meanings to botanical terms and to discuss them in orderly fashion. Rapid adoption of his system of classification led also to wide use of the vocabulary defined by him. It is in this sense that we may speak of 1751 as the beginning of our use of taxonomic terms.

In the "Philosophia" (p. 37) the parts of a plant are named Radix, Herba, Fructificatio. Under Herba we find the subdivision Truncus (p. 39), under this in turn Pedunculus (p. 40). Peduncles are classified first by position (radicalis, caulinus, axillaris, *etc.*); then by the manner in which the flowers are borne (modo quo flores gerit & connectit summitate). Under this head (p. 41) are such divisions as uniflorus and biflorus; and terms referring to the arrangement of flowers on the peduncle: fasciculus, capitulum, spica, corymbus, panicula, thyrsus, racemus, verticillus.

The definitions of the latter series of terms are worth noting. A fasciculus holds its flowers erect, parallel, in a bundle, as in *Dianthus barbatus* (now known to be cymose). A capitulum has flowers congested into a firm globe. A spica bears sessile flowers scattered on a common peduncle; it may be secund or distichous. A corymbus arises from a spica when the flowers are pedicellate[1] in proportion to their position on the peduncle (as in *Sedum* and the "Siliquosae"). A panicula is a sparse cluster differently subdivided into peduncles; it may be diffuse or coarctate. A thyrsus is a coarctate panicle of ovate form (*Syringa, Petasites*). A racemus has short lateral branches on the peduncle. A verticillus consists of numerous subsessile flowers surrounding the stem in a ring. Plate 9 illustrates the corymb (fig. 163), the raceme (fig. 164), the spike (fig. 165—the compound spike of a grass), the verticel (fig. 166—a mint?), and the panicle (fig. 167—another grass).

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[1] The word petiolis is used in the "Philosophia", doubtless a misprint for pedicellis.

The omission of umbels and cymes from this list is at first puzzling. The solution is found on pp. 54 and 55 under the heading Receptaculum. The receptacle is the common base to which the parts of the fructification are attached. Receptaculum proprium bears the parts of only one flower, receptaculum commune bears several florets (as in Compositae). [For this facet of Linnaeus' thought, see also the "Fundamenta botanica" (68).] An umbella is a receptacle of filiform peduncles arising from a common center; it may be simple or compound. A cyma is like a compound umbel, the primary divisions arising from one point; but the secondary are scattered. A spadix is the receptacle of the palms, which arises from a spathe. On p. 87 flowers and their parts are further expounded. A flower may be simplex or aggregatus; of the latter kind Linnaeus recognized seven types: umbellatus, cymosus (*Cornus sanguinea*), aggregatus properly speaking (*Scabiosa*), amentaceous, compositus, glumosus and spadiceus. The spadix as treated here is either simple or branched; the former includes *Calla, Arum* and *Zostera,* the latter the palms.

It is strange for us to consider inflorescences under two heads, since we think of them as branch-systems, all related in the sense of being derivable from one underlying type. But, after all, flowers themselves may be branch-systems, and perhaps Linnaeus anticipated a modern view in failing to distinguish between single flowers and close aggregates of flowers. The same approach is evident in his treatment of the calyx (p. 52), which might enclose a single flower or many, and whose several varieties were perianthium, involucrum, amentum, spatha, gluma. It is noteworthy that Linnaeus did not include either peduncles or receptacles under a heading "inflorescences"; inflorescentia was to him not a branch, not a structure, but the manner of flowering—modus, quo flores pedunculo plantae annectuntur (p. 112). It is indeed possible that in reducing all so-called inflorescences to a common status we are oversimplifying: they may have originated in several ways. But Linnaeus' effort, which was first to name them descriptively, was only confused by the division which he made.

Linnaeus' botanical terms appeared separately in a dissertation published in 1762 (71).[2] This work corresponded closely with the treatment in the "Philosophia" except for the transfer of the term *inflorescentia* to the discussion of peduncles. It went through several editions and versions without substantial change. Gilibert's edition of 1786 (33) and that of Rotheram in 1789 (94) were essentially identical with the original. Giseke's version of 1787 (34) was considerably enlarged and included a German translation parallel with the Latin. In this work cyme and umbel joined company with raceme, panicle and the rest under inflorescentia.

The "Philosophia", one of the first botanical textbooks, enjoyed a deserved popularity. It was translated into French, German, Spanish and English, besides passing through several editions in Latin. Lee's "Introduction to botany" of 1760 (61), which itself appeared in nine editions, included a nearly literal translation of many parts of the "Philosophia" in a reorganized arrangement. Rose's "Elements of botany" (93), published in 1775, was a nearly literal version, in English. As late as 1812 and 1818 Thornton's "Elements of botany" (104, 105) contained what was essentially a translation of the Linnaean vocabulary.

Other elementary treatises were appearing during the same period. In 1764 and 1766 Oeder (84) brought out his "Elementa botanica" (a German edition appeared at the same time under the title "Einleitung zu der Kräuterkenntniss"). Chapter 3 was devoted to terminology. The treatment is essentially Linnaean; but umbella finds its place among the other modos florendi. (The cyma seems to have been accidentally omitted; it is included without definition in the "Index alphabeticus terminorum technicorum.") Scopoli, in his "Fundamenta botanica" of 1783 (100), has a section devoted to the inflorescence (still a mode of flowering), in which we find most of the Linnaean terms; the cyma is here merged with the corymbus. Willdenow's "Grundriss der Kräuterkunde" of 1798 (109) has a similar chapter on terminology.

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[2] It is difficult to decide on the authorship of Linnaean dissertations. The usual view is that they were written by Linnaeus and defended by the candidate (in this case Elmgren). There is, however, internal evidence in some of them that the dissertation was really the work of the student. In any case, this one was entitled "Linnaeus' botanical terms", and adopted as such by its various subsequent editors.

Meanwhile botanical dictionaries were making their appearance, differing from the more "philosophic" works in their alphabetic arrangement and in including, besides definitions of Latin terms, the same concepts expressed in modern tongues.

Of these we may note Berkenhout's "Botanical lexicon" in 1764 (8), the "Botanical dictionary" by Milne in 1770 (77), Bulliard's "Dictionnaire élémentaire de botanique" of 1783 (15), Martyn's "Language of botany" of 1793 (74), Jolyclerc's "Principes" of 1797 (56), Borckhausen's "Botanisches Wörterbuch" of 1797 (10), and Frege's "Versuch" of 1808 (30). Little or nothing new was introduced into these lists; all are substantially alike, the later evidently founded upon the earlier. One of the most sumptuous of early botanical dictionaries was that by Hayne (47) which includes perhaps the first effort towards color standardization. Such glossaries as that of Leers in the "Flora herbornensis" (62), that in Withering's "Botanical arrangement" (113), and that in the third edition of Lee's "Introduction to botany" (61) are evidently also based on the current dictionaries. The Linnaean system of terms persisted well into the nineteenth century, as in the "Dictionnaire raisonné" of Gérardin and Desvaux (31), and in American textbooks (5, 23).

The eighteenth century, then, which saw the rise of the Linnaean system of classification, added little to the vocabulary defined by Linnaeus. Minor changes of meaning appeared, as when Bulliard defined inflorescentia as synonymous with efflorescentia or floraison:—"c'est l'époque à laquelle les plantes portent des fleurs". The classic meaning of cyma, a sprout, tuft, or the tip of a plant, crops out here and there (Bulliard, Jolyclerc, Withering). Similarly, Berkenhout wrote: "Corymbus, in its proper acceptation is a cluster of ivy-berries. Linnaeus makes it . . .". Bulliard is unique in separating true and false umbels on the basis of the fruit which they bear—a true umbel having the characteristic fruit of the Umbelliferae. The difficulty introduced by Linnaeus in his division of inflorescences between peduncle and receptacle soon was evident, and the proper solution accepted. Milne remarks "Cyma, a species of receptacle, according to Linnaeus; or more properly, a mode of flowering, in which, like the umbel . . .".

A PHILOSOPHY OF INFLORESCENCE: ORDER OF FLOWERING

The names of inflorescences used by the systematists of the eighteenth century were based on fairly obvious features, chiefly the shape of the entire cluster and the mode of arrangement of branches upon the primary axis. It was a useful and workable system but of course did not offer much opportunity for fine distinctions; and, lacking the morphological ideas now prevalent, was not adequate for a science which had to extend itself constantly and finally to adapt itself to a less static system of nature.

Near the turn of the century changes began to appear almost simultaneously and independently in France and in Germany. Bulliard's "Dictionnaire élémentaire" was revised by Louis Claude Richard (89); his edition appeared in 1798.[3] In this the "Dictionnaire des termes latins" remained substantially unchanged, but the part in French was entirely rewritten and new terms added. At the end of the dictionary proper was "Esquisse d'un vocabulaire méthodique de botanique en seize tableaux á l'aide desquels, et du Dictionnaire, l' Étudiant peut prendre une leçon suivie sur chaque partie des Plantes". Tableau 8 treated the disposition of flowers. The Linnaean vocabulary is enlarged by the addition of sertule or sertulum (p. 133, 183), a sort of sessile and simple umbel; cephalanthe (p. 184), the head of Compositae; periphoranthe (p. 184), the involucre of Compositae; phoranthe (p. 185), the receptacle of the cephalanthe.

Richard apologizes for the new terms introduced and expresses a wish that others would be substituted (p. 157). In 1802 Mirbel, however, in the "Traité d' anatomie" (78), kept closely to the Linnaean scheme, and in 1815 in the "Élémens de physiologie végétale" (79) introduced only one new term for an inflorescence: calathide or calathidis [more properly calathis] (p. 284, 778), equivalent to Richard's cephalanthe. The peduncle which supports a calathide was termed a clinanthe (clinanthium; p. 273, 752). In 1819 Achille Richard published his "Nouveaux élémens de botanique" (88), in which he maintained the terms introduced by his father. In the same year appeared one of the first attempts at morphological study of inflorescence, Turpin's "Mémoire" (106).

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[3] Bulliard died in 1793. In 1797 his publishers brought out a second edition of his work, and in 1812 a third; these were practically identical with the first edition, and the third took no cognizance of Richard's revision.

While French scientists were working within the Linnaean tradition, so far as concerns this subject, nothing less than a revolution was occurring in the concepts of inflorescence held in Germany. A characteristic tendency towards romanticism had asserted itself in the application to scientific terminology of poetic and philosophic concepts. Since we are still involved in the resultant muddle, it is perhaps worth while to trace the beginnings of this Germanic contribution to botanical thought.

Linnaeus in 1760 (70)[4] had proposed his curious theory of prolepsis (anticipation). He called attention to the fact that in the axil of every leaf appears a bud, which when it expands is seen to have leaves in whose axils are buds; and so on . . . "quosque se extendat nemo facile dixerit". On a tree each set of buds normally represents a year's growth; but under certain circumstances the opening of the buds may "anticipate" the proper season, and next year's leaves appear this year. He further adduced evidence, largely teratological, to show that the parts of flowers were really leaves, changed in form and less expanded. Now since each flower, he argued, arises from a bud in the axil of a leaf (bract), it must itself be a secondary branch bearing leaves in whose axils are buds, and so on. The calyx was conceived as the leaves of the secondary branch. The buds in the axils of the calyx-leaves formed the third series, the petals (perianthium). The leaves of the fourth and fifth sets were stamens and pistils, respectively. Adhering to his primary assumption about buds, Linnaeus maintained that each of these series represented a year's growth, which because of nutritive conditions had anticipated their proper seasons and appeared all together. Or, to look at it in a different way, the primary leaves in any bud are foliage leaves; the secondary are potentially bracts, but may expand into foliage under suitable conditions; the tertiary are calyx, with the same restriction; and so on. A leaf-rudiment might expand either during its appointed year as a leaf, or precociously as a flower-part. The theory is developed with considerable ingenuity and fortified by a wealth of observations on plant life. Naturally it is closely associated with the physiological ideas then current. The latter aspect was particularly emphasized in another dissertation, with almost the same title, which appeared in 1763 (72).

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[4] In a dissertation by Ullmark under his supervision. See note 2.

The hypothesis in Goethe's hands was the basis of the celebrated "Versuch die Metamorphose der Pflanzen zu erklären" of 1790 (37). Goethe is often credited with having originated the theory of metamorphosis; this is obviously an error. He did, however, inject into it a considerably greater philosophic flavor, and his eloquence and prestige gained it a wider hearing than it might otherwise have had. Though few botanists have read the "Versuch", it still dominates their thoughts.

Goethe was preoccupied with the ends and purposes of life rather than with its nature and mechanisms. Metamorphosis was to him a progress from the level of the seed to the perfection realized by the flower—sexual reproduction being the aim of the plant.[5] Since the upper nodes grew from the lower, they received their sap filtered and perfected, and so each succeeding set of leaves tended more towards the glorious consummation.[6] On this basis he developed a classification of the factors in metamorphosis which result in the different parts of the flower, and was confident that he had discovered a simple key by which all the secrets of form in the plant world could be unlocked.[7]

The consequences of this sort of nature-philosophy are seen in Link's "Philosophiae botanicae novae prodromus" of 1798 (65), the dogmas and definitions of which were further expounded in his "Grundlehren der Anatomie und Physiologie der Pflanzen" (66) and again in the "Elementa philosophiae botanicae" (67).

It is in Link's works, apparently, that the first attempt was made to introduce the order of flowering into the classification of inflorescences. In 1798 this appeared simply as an aid to description. He introduced a complex system of terms, *e.g.:* inflorescentia centriflora, divided into inflorescentia centrica and inflorescentia centralis; inflorescentia eccentrica (and eccentralis and subeccentrica); and

[5] ". . . durch Umwandlung einer Gestalt in die andere, gleichsam auf einer geistigen Leiter, zu jenen Gipfel der Natur, der Fortpflanzung durch zwey Geschlechten hinaufsteigen" (p. 3).

[6] ". . . ein oberer Knoten, indem er aus dem vorgehenden entsteht und die Säfte mittelbar durch ihn empfängt, solcher feiner und filtrierter erhalten, auch von der inzwischen geschehener Einwirkung der Blätter geniessen, sich selbst feiner ausbilden und seinen Blättern und Augen [buds] feinere Säfte zubringen müsse" (p. 18).

[7] "Wir sind überzeugt, dass mit einiger Uebung es nicht schwer sey, sich auf diesen Wege die mannigfaltigen Gestalte der Blumen und Früchte zu erklären; mir wird freylich dazu erfordert, dass man mit jenen oben festgestellten Begriffen der Ausdehnung und Zusammenziehung, der Zusammandrängung und Anastomose, wie mit algebraischen Formeln bequem zu operiren, . . ." (p. 67, 68).

inflorescentia basiflora; terms which were apparently never used by others to any extent, and which involve distinctions between "rami veri" and "ramuli" which now seem fanciful. The inflorescence, incidentally, was to Link a "complex of peduncles" rather than a mode of flowering. In his later exposition of this system of names, Link developed a unique theory of growth. He acknowledged his debt to Linnaeus in suggesting the homology of leaves and flower-parts, but rejected the "prolepsis" theory. One flower and all its parts belong to one bud. He pointed out that in a single shoot the opening of axillary buds during the season of growth is acropetal. Such a system he regarded as a single "ramification" and the growth of a single bud (66, p. 172).[8] On the other hand, when the buds on a woody twig open, the terminal usually blooms first, the subterminal next, and so on; the opening is basipetal (66, p. 174).[9] This, then, was not a single ramification but a group, each bud being a unit in itself. It is unnecessary to point out the flaws in this theory; they are suggested by ordinary observation. It is sufficient to quote a few passages to show the consequences of these axioms. "Most spikes and racemes bear flowers of one bud and open from below up. In contrast the panicle displays flowers of several ramifications—sometimes each flower belongs to a distinct ramification" (66, p. 175).[10] "Herein also the umbel is distinguished from the cyme; in the former all flowers are of one ramification, the outermost or lowermost flowers bloom first; in the latter the flowers belong to different ramifications, the central flower blooming first between two side flowers" (66, p. 175).[11] We must regret that Link's keen observation did not yield a more scientific classification.

Having rejected "prolepsis" within a single flower, Link then adopts it as applied to buds, and speaks of each order of buds as representing a year's growth, and the blooming of a centrifugal inflorescence, therefore, as indicating an anticipation of many years.

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[8] "Zu einem Aste, oder zu einer Verästelung gehört Alles, was aus einer und derselben Gemme erwachsen ist".

[9] "Es ist die Regel, dass der Hauptstamm früher blühet, als die Aeste".

[10] "Die meisten Aehren und Trauben tragen nämlich Blüthen einer und derselben Gemme und blühen von unten auf; hingegen bringen die Rispen gewöhnlich Blüthen verschiedener Ramificationen hervor, und oft so, dass jede Blüthe zu einer andern Ramification gehört".

[11] "Dadurch unterscheidet sich auch die Dolde (umbella) von der Afterdolde (cyma); in jener gehören alle Blüthen zu einer Ramification, die aüsseren und eigentlich unteren blühen zuerst und so fort, in dieser aber gehören sie zu verschiedenen Ramificationen, es blüht immer die Mittelblüthe zwischen zwey Seitenblüthe früher, als diese letzteren".

Anthodium had been proposed by Ehrhart (28, p. 64) for the flos compositus of Linnaeus, and applied to the heads of Compositae. Link adopted this term and recognized five subdivisions (67, p. 258): umbellula (Umbelliferae); spicula (Gramineae); calathidium (Compositae); amentum; strobilus; hypanthodium (*Ficus, Dorstenia, etc.;* equivalent to the amphanthium of the "Prodromus"). Willdenow had misapplied the term anthodium (109, p. 84) to the "allgemeine Blumendecke" (involucre) of Composit Another term introduced by Link is anthurus for a contracted panicle.

It is interesting that at this same time Robert Brown (13) made use of similar ideas to explain the centrifugal flowering of *Lagascea* and *Echinops.* The Compositae, to which these are evidently related, flower from the margin to the center of the head; but heads are frequently aggregated in "cymose" clusters which come into bloom in the opposite order. The "head" of *Echinops* is then not a head but a cluster of one-flowered heads. This explains also the "outer perianth" which had puzzled the early observers; it is an involucre. It will be noted that Brown's adoption of such a method of deduction is based not upon philosophical principles but upon morphological data. He does not acknowledge any debt to his German contemporaries, and it is possible that the resemblance of ideas was fortuitous.

The new terms proposed by Link were included in Illiger's "Terminologie" of 1800 (53) in a rather confused way; but only at the hands of Roeper in 1826 (92) did a system based on the order of flowering attain some degree of clarity and usefulness. His "Observations sur la nature des fleurs et des inflorescences"[12] betrays the same preoccupation with metamorphosis as the writings of his predecessor; the doctrine is here revealed as firmly entrenched, almost axiomatic, in botanical thought. Since carpels are leaves, argues Roeper, they cannot really terminate the axis; they only seem to—because they use up the "nutrient sap".[13] Hence flowers are not really terminal structures, though they appear to be.

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[12] Published also in Latin shortly after the appearance of the French version.

[13] The pioneers in botanical thought, from Linnaeus to Link, fortified the weaknesses in their systems—of which they were aware—with appeals to current physiology. It is disquieting to realize that today, with a much ampler and quite different physiology at our command, which would not support their conclusions, we are apparently so uncritical as not to realize the need for fortification of the same questionable assumptions.

Roeper homologizes the flower with a leafy branch; both arise in the axils of leaves. He notes the bracteoles (later called prophylls) on both. He passes to a comparison of the inflorescence (here distinctly a flowering branch, not a mode of flowering) with a single flower. It is curious, now that we are getting evidence that a stamen may have been derived from a branch (110), to read his comparison of stamens and branches; though he hastens to assure us that the resemblance is purely a matter of disposition and that the structures are not homologous. It should be clearly borne in mind that homology to these botanists was a pre-evolutionary concept. It did not refer to relatedness due to descent, but harked back to some more or less mystical "order of nature."

It is against such a fanciful and philosophic background that we find our familiar inflorescence-types arranged for the first time in the "modern" groups determinate (definite) and indeterminate (indefinite). In the first are glomerule, cyme and fascicle; in the second spike, ament, spadix, raceme, corymb, umbel, capitulum, panicle and thyrse. The new criterion at once found a place in current botanical works, as Keith's "Botanical lexicon" (59).

The next landmark in this progress is A. P. de Candolle's "Organographie végétale" (17), published in 1827. The same author's earlier "Théorie élémentaire de la botanique" (16)[14] had adhered closely to the Linnaean terminology. But in the interval de Candolle swallowed the Link-Roeper system hook, line and sinker—and in the "Organographie" regurgitated it at considerable length. He develops the fundamental theme that flowers originate in the axils of leaves, the latter often indistinguishable from ordinary foliage leaves, and continue to appear acropetally as the stem grows from its apex. This is the indeterminate or "axillary" inflorescence. Bracts he thought of as undernourished leaves. In the alternative method the stem terminated its growth with a flower, and further growth occurred subsequently from leaf-axils beneath it. It seemed not to worry him to have two distinct methods of growth among closely related plants—as it has not worried us since.

De Candolle, however, realized—as his predecessors had not—that the simple system which they had created was insufficient for a description of the facts. He distinguished the "cime scorpioide"—the first step towards recognition of a sympodial axis. He called attention to the rarity of true verticels, showing that the labiate inflorescences usually so described are really contracted cymose clusters—the verticillasters of Bentham (7). This had been recognized previously by Nees von Esenbeck (83). When a series of such clusters, by modification of the subtending leaves and shortening of the internodes, forms one inflorescence, the name thyrse was applied to the whole; the thyrse is therefore, in this system, not indeterminate but "mixed". De Candolle recognized that the thyrse might be reduced to a raceme- or spike-like form or to a very small cluster; he here foreshadowed a view of inflorescences which invalidates much of his classification of them. The converse of the thyrse was the corymb, which, as he proposed to use the word, referred to indeterminate clusters arranged in a determinate system, examples being adduced from the Compositae. The panicle, exclusive of many inflorescences called panicles but more correctly referred to the thyrse, remained a strictly indeterminate system, a compound raceme. The anthela of Meyer (76, p. 11) was adopted in the sense of a panicle with a short primary axis and long branches.

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[14] A third edition appeared in 1844, like the first two in text but with notes by Alphonse de Candolle embodying the ideas of the "Organographie".

Botanical terminology of the first quarter of the nineteenth century was ably expounded in Bischoff's great "Handbuch der botanischen Terminologie und Systemkunde" (9), which appeared from 1830 to 1844. Bischoff adopted the ideas of Link and of de Candolle, and added considerable discussion of his own, especially developing the concepts outlined in the preceding paragraph. Thus early was the realization attained which has been strangely neglected in our times, that spikes and racemes are not the simple structures called for by the definitions current then and now.

PHYLLOTAXY AND SYMPODIA

The next inspiration in the study of inflorescences came from the investigation of phyllotaxy, developed principally by Schimper and Braun in 1835[15] (11). It is unnecessary here to follow these authors through the devious arithmetical progressions in terms of which they attempted to elucidate the laws of plant growth. Their importance for us lies in the emphasis upon the prophylls[16]—the first leaves of a branch and therefore the beginnings of new spirals of leaves. Braun pointed out (p. 189) that in both axils of the prophylls (Vorblätter) beneath a flower branches may arise which themselves bear flowers; the result is the structure named by Schimper a dichasium. If, however, only one axil bears a branch, two types of inflorescence are possible, according as the succession of branches is homodrome or antidrome. If each successive fertile axil bears such a relation to the preceding that a continuous spiral could be drawn through them, we have an inflorescence which Schimper called a bostryx, the helicoid cyme of later authors. If, on the other hand, the line from bud to bud along the (false) axis reverses itself at each axil, the result is what de Candolle had named the scorpioid cyme, or what Schimper named the cicinus. So with this Schimper-Braunian movement we are for the first time immersed in the vast tangle of inflorescences whose axis is sympodial—made up of successive axillary shoots whose terminal extension is always limited by a flower or a flower-rudiment.

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[15] Schimper's proposals were made in a scientific meeting at Stuttgart. They are elaborated by Braun in the work cited. For the reasons that Schimper failed to publish his own ideas, see Flora **18**: 737–758 (21 D 1835). Schimper expounded his phyllotaxy in another paper appearing in 1835 (97).

Two years after this notable contribution from Germany there appeared a monumental work by two Frenchmen, the brothers Louis François and Auguste Bravais (12). It is remarkable to what an extent these men, whose researches had evidently occupied several years, had reached the same concepts as Schimper and Braun; a footnote (p. 195) makes it clear that their work was quite independent. Like their German colleagues, they were preoccupied with the spiral disposition of leaves and its arithmetical notation. The complex and perhaps too rigid system which they developed was applied very thoroughly to the description of inflorescences—with rather surprising results.

Hemerocallis and *Alstroemia* have a "uninodal" helicoid cyme; *i.e.,* beneath each flower is a single node (we should say axil; see below) from which grows the new peduncle. The sympodium formed of all the successive peduncles was designated the pseudothalle; each individual segment was a mérithalle. This is exactly the bostryx of Schimper and Braun; and, exactly like their cicinus, the uninodal scorpioid cyme is derived by a reversal of the direction of the spiral at each "dichotomy". If the "mérithalles" of a helicoid cyme are very short, we have the sort of axillary cluster seen in *Yucca gloriosa* or the terminal cluster found in *Amaryllis, Allium* and *Narcissus*—"et probablement de la plupart des sertules analogues qu'il avait paru naturel aux botanistes de rapporter à l'inflorescence centripète". Likewise the scorpioid cyme, besides yielding the clusters of *Lamium, Musa* and other genera, could be easily mistaken for a distichous spike. Here is the first clear indication of the prevalence of the "cymose" or sympodial type of inflorescence; and though the authors do not actually express a suspicion that most "indeterminate" inflorescences are misinterpreted and misnamed, the amplitude of their examples and the ingenuity of their interpretations must arouse such a suspicion in the mind of the reader.

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[16] The word prophyllum seems to have been first used by Wydler (118, p. 292), though by him it was attributed to Schimper.

In the dicots two prophylls are usually present. Here the doctrines derived from phyllotaxy are intruded. Even when the two bracts are opposite, one is considered to be "organically" upper, the other lower, so that "la spire génératrice" can pass through these two "nodes" successively. There is in fact frequently a slight or marked difference between the two bracts, in position, size or form. Cymes of dicots are therefore binodal (in the usual modern sense they often have one node beneath each flower, with two axils). If only one of the nodes (axils) yields a branch, the cyme is uniparous (unipare). Such distinctions are as valid today as then, though the terminology may be unnecessarily elaborate. It is unnecessary for us here to follow the authors in their distinction between ascending and descending cymes, which referred to the position (upper or lower) of the fertile node at each joint of the sympodium. The cime binodale unipare is common, often in contracted form. The cime binodale bipare (both axils fertile) is the dichasium of Schimper. The authors discuss at length the morphological peculiarities of these inflorescences, the coiling of the sympodium, the inequality of the bracts and their union with the peduncles which they subtend, the different ways in which the cymes may be expanded or contracted, trinodal and multinodal cymes (trichasia and pleiochasia of a later terminology). A startling innovation—made only in passing—is the proposal to define raceme as a multiparous cyme—"car tel est, par le fait, le vrai sens linnéen de ce mot . . .". The multiparous cyme can also imitate a corymb or a panicle. The first is seen in *Cornus sanguinea* (the

Linnaean cyma), *Viburnum, Sambucus, Crataegus, Tilia, Sedum telephium, Spiraea;* the second in *Polemonium, Melia, Syringa, Ligustrum, Cornus paniculata.* Another variety of this class is that which imitates a spike with a terminal flower, as in *Berberis, Poterium, Campanula, Anemone;* this was named cime spiciforme, and the term corymb was also applied to it. A thyrse is taken in the Candollean sense—a series of contracted cymes arranged acropetally along an axis. The Bravais' sarmentide was something like de Candolle's corymb—a group of clusters (of any type) arranged as are the single flowers of a cyme. As for panicle, they understood by this name any compound inflorescence whose flowers do not form a definite surface. The classification of compound clusters involved the Bravais' in an attempt to delimit the inflorescence from the rest of the plant; they remark that one may be astonished that at the end of their work they tackle the question: "What is an inflorescence?" After noting the two senses in which the word had been applied, they point out that to classify inflorescences as centrifugal or centripetal is inexact. The order of opening of the flowers may indeed be thus characterized; but when one says *centripetal* one is referring to *branches of the same order* (all arising from one common axis), while when one uses the word *centrifugal* one is speaking of *branches of different orders* (one arising from another). Most inflorescences—even a biparous cyme—involve both these characters. A flash of insight which, like their other more audacious ideas, the Bravais' did not fully exploit. Note the difference between this matter of simple observation and Link's theoretical distinctions between "ramifications".

No account of the botany of the early 19th century can omit Schleiden. In his treatment of inflorescences (98, p. 224, 225) he characteristically points out the inconsistencies in current treatments, brands most of the terms in use as illogical and unnecessary, and laments the lack of morphological study. He further comments on the insufficiency of one arbitrary distinction to separate two groups of structures. Characteristically also he denies that the bostryx and cincinnus are really sympodial, asserting that they are merely one-side racemes. It is a pity that Schleiden, the iconoclast, so often attacked truth instead of error; if he had chosen to uproot the Goethean doctrines, which he tacitly adopted, instead of the valid concepts of inflorescence, his criticism would have been more significant.

DEVELOPMENT OF MODERN TERMINOLOGY

The further history of the study of inflorescence is largely the efforts of botanists to use the systems bequeathed to them. The distinction of two groups of inflorescences became traditional. Saint-Hilaire, indeed, suggested in 1840 that because of the variety of inflorescences that had been called cyme, it might be best to abandon the term (96, p. 310). He made an effort to derive all inflorescences from leafy branches terminated by flowers (p. 276), an idea destined for later exploration. The unifying trend of his thought is seen in his transfer of the cicinus to a "racemus scorpioides" (p. 320).

The characteristic exponent of inflorescences was Wydler (116–119), who, complaining of the current neglect of the subject, set out systematically to catalogue the inflorescence-types in as many families of plants as he could investigate. His papers are still valuable references for such data. He varied the accepted system slightly by grouping inflorescences in three types: racemose (aehrige), paniculate (rispige) and cymose (gabelige, dichotome). This was evidently an attempt to fashion a more purely descriptive terminology. A number of inflorescences were described also by Irmisch (54); his only contribution to the general concept was to distinguish a flower-cluster as terminal—no matter what its position—if it possessed leaves in addition to the prophylls. Ascherson (3) presented inflorescences in the form of a key. Buchenau (14) added the terms rhipis (Fächel; later called rhipidium) and drepanum (or drepanium; Sichel) to the increasingly elaborate vocabulary of "cymose" types. A rhipis is a bostryx in which all the flowers are in one plane; a drepanum has a like relation to a cicinus. Hofmeister (51), though giving a conventional account of inflorescences, redefined the cymose type as that in which the side branches exceeded the main axis in branching—a symptom of the increasing difficulty that was experienced in explaining the real difference between the two types. In the same year (1868) appeared the first edition of Sachs' great textbook (95), in which the words monopodial and sympodial are used for the distinction. In the second edition the account was rewritten, without any great gain. The terminal flowers of the racemes of *Berberis, Menyanthes* and *Campanula* are noted—and invalidate the preceding discussion. The anthela is transferred from the monopodial to the sympodial group, without apparent reason. In this edition also he changed his view on the occurrence of "true dichotomy" in the flowering plants—evidently because of the work of Kauffmann (58) and of Kraus (60) in 1871. The idea that the "cymose" inflorescence was produced by dichotomy of the growing point had a period of prosperity, being accepted also by Warming (108). It was discarded by Eichler (29) in 1875, and has remained discredited since then, perhaps on insufficient evidence. There was little detailed anatomical work on inflorescences in the 19th century; Hieronymus made a beginning in 1872 and 1886 (49, 50), Goebel in 1884 (35), and Schumann in 1889 (99), but this field remains relatively untilled to the present day. Grégoire (42) has recently described and figured what he considered dichotomous branching of the axis of the inflorescence in *Spiraea* and *Sambucus* (see p. 337, 338; pl. 3, f. 29; pl. 4, f. 37).

Meanwhile in France Guillard (43–46) had written at some length on inflorescences, replacing several current terms with new ones of his own which have sunk into a merited oblivion. Credit is due him, however, for calling attention (p. 35, 36) to the order of flower-opening in *Rubus* and elsewhere, in which the first flower to open is the terminal, after which flowering is from the base upwards. Guillard distinguished between the mode of flowering, for which he retained inflorescentia in the classic sense, and the system of flowering branches, for which he proposed the name anthémie. He gave also a very complex terminology for compound inflorescences (44, p. 377–380). Actual practice in France may be judged by Germain de Saint-Pierre's "Nouveau dictionnaire" (32), a simplified version of the Bravais' proposals.

The contributions of the 19th century to this subject were admirably summed up by Eichler in the "Blüthendiagramme" (29). He retained the now established grouping of types, calling them botrytischen (botryose) and cymösen (cymose); but he admitted that they corresponded to no sharp line in nature. He pointed out the absurdity of following the Bravais' in adopting the presence of a terminal flower as criterion, since the raceme of *Berberis* sometimes has one and sometimes not—both conditions being found on a single branch. Moreover, many cymes lack a terminal flower. It is useless to rely on the prophylls, for cymes may flower also from other leaves. The order of flowering is likewise deceptive, for the end-flower of a raceme may open first. Eichler's own distinction (admittedly imperfect) was in the relative development of main axis and lateral branches.

In his systematic key Eichler gave a complete account of all inflorescences known to him, classified in the accepted terminology. He introduced the artificial separation of "simple" from "compound" inflorescences (with unfortunate consequences, as will be seen). Of botryose types he mentions the spike, raceme, umbel, head, *etc.*—"auf deren Charakteristik ich wohl nicht einzugehen brauche". Of cymose types he recognized the pleiochasium (cime multipare), the dichasium (cime bipare) and the monochasium (cime unipare). Among the latter he distinguished bostryx and cincinnus, rhipidium and drepanium. Phyllotaxy was not invoked in the explanation of these types. The supposed dichotomy of certain cymose types was examined and discarded. Compound inflorescences were rather elaborately treated under such terms as Dibotryen, Cymobotryen, Botryocymen, and Dicymen, with abundant examples. Such words as Rispe (panicle) he would drop because they described only external form, not real structure.

A paper by Hy in 1894 (52) contributed little. The many terms introduced were unfortunately based largely on misunderstanding (*e.g.*, the inflorescence of *Gladiolus* is described as racemose). He did note, however, that most branches of the second order in compound inflorescences are cymose—a pregnant observation.

In England, in spite of the activities of Robert Brown, interest in this subject lagged and understanding of it was correspondingly deficient.

Lindley in 1832 (63) explains a cyme as follows: "Suppose the branches of a deliquescent panicle to become short and corymbose, with a centrifugal expansion indicated by the presence of a solitary flower seated in the axillae of the dichotomous ramifications, and a clear conception is found of what is called a cyme". This adds only verbiage to Linnaeus' definition of 81 years before, except for the grafting onto it of an idea from Link. And this paragraph survived Roeper, Schimper and the Bravais', persisting into the fourth edition of 1848. To Lindley all inflorescences were derived from the raceme. "A panicle is a raceme, the flower-buds of which have, in elongating, developed other flower-buds" (64, p. 39). With such unclear teaching as this in a leading textbook, it is small wonder that English botanists failed to grasp current ideas. Paxton, in his well known botanical dictionary (86), gave definitions of inflorescences which were essentially 100 years old and 14 years out of date; these were unchanged in subsequent editions. Likewise in the various editions of Smith's "Botany" (101) the original Linnaean treatment remained unchanged.

Both Henfrey in 1847 (48) and Balfour in 1849 (4) confused scorpioid and helicoid cymes, apparently not having carefully studied either the plants that bear them or the writings of continental botanists. Henfrey speaks of the "true cyme, found in all species of *Viburnum, Sambucus, etc.*" (p. 133). Balfour expounds the familiar doctrine that flowers, being branches derived from buds, are naturally found in the axils of leaves, which are (later) metamorphosed into bracts. As late as 1862 Cooke (24) could still define a cyme as "a kind of inflorescence developed in a centripetal manner", without further exposition, and a cincinnus as "a cyme developed in a scorpioid manner" (the figure, however, shows a drepanium); the bostryx is lacking. An understanding of the sympodial nature of the axis of such flower-clusters seems strangely absent, a deficiency which contrasts with the active discussion taking place in Germany during the same period. It was not until 1900, with the publication of Jackson's well known "Glossary of botanic terms" (55), that the current concepts of inflorescence achieved recognition in English. Even a recent work on "floral mechanism" (57) contains an account of helicoid and scorpioid cymes which betrays a remarkable lack of grasp of the essential concepts.

In this country also the study and the understanding of inflorescences languished unduly during the last century. There are signs that this was connected with the development of the characteristic American textbook. The spread of popular education caused the multiplication of texts, which were most easily compiled by copying from their predecessors, and which had to be kept to an elementary treatment (characters which persist to this day). The earlier ones, by Barton in 1803 (5), Eaton in 1829 (27), Comstock in 1833 (23) were, of course, essentially Linnaean. Later editions of such early classics, from 1836 even to 1870 (23), merely reprinted the original discussion of inflorescences, no matter how out of date. The limited progress made by American botanists in adapting themselves to the ideas developed in Europe (ideas to which they did not themselves contribute) is best seen in the classical works of Asa Gray. His early textbook of 1836 (38) was apparently well received from the first. Yet the discussion of inflorescences therein shows merely that he had seen the works of European botanists without having grasped their ideas. "Determinate" and "indeterminate" are adopted without discussion and without reason—without reference to phyllotaxy, or to any current theories of nutrition or of the different orders of branching; prophylls are not mentioned as such, and the different sorts of bracts are evidently confused. In short, this was an oversimplified treatment designed only for the classroom. Incidentally on page 171 the corymb is apparently determinate, though on page 174 it is clearly indeterminate.

In the textbook of 1842 (39) we find a hint (p. 97) of the existence of various sympodial inflorescences: "Various modifications of both forms of inflorescences, but especially of the centrifugal or cymose, arise from irregular developement . . . : but the student who clearly understands the *normal* or regular arrangement, will generally be able to comprehend the irregular deviations. For obvious reasons, a regular centrifugal inflorescence is very seldom met with except in plants with opposite leaves; and in these it is more common than the centripetal mode". Gray was as optimistic as Lindley on the comprehension of his readers. He must, however, be given the credit of pointing out (in the last sentence quoted above) that the "cymose" types, which have been given relatively scant treatment in most textbooks (including his own), are actually predominant in large and familiar groups of flowering plants.

In the edition of 1845 the "scorpioid or helicoid cyme" is introduced; the two confused, in a wholly inadequate discussion. This treatment lasted until 1877 (40), where we still read of "several abnormal modifications of definite inflorescences". The figures of cymes are merely diagrammatic. The obviously determinate clusters of *Cornus* and *Asclepias* still exemplify the indeterminate head and umbel, respectively; the thyrse of *Syringa* is called a panicle, which is classed as a "racemose" type. And this long after Roeper, the Bravais', Saint-Hilaire, Wydler, Buchenau and Sachs had published their researches on inflorescences!

Finally in 1879 (41) Gray came out with a revised system, more or less up to date, which he called the system of Eichler. The terms botryose and cymose were adopted, also sympodial. *Cornus* is transferred from the indeterminate heads to the determinate glomerules (but *Asclepias* stays unbellate). The term fascicle is used in both groups. Scorpioid and helicoid cymes are distinguished only in a footnote. In a footnote also (p. 155–157) the whole summary of Eichler is reproduced. Small wonder that the American student, despairing of finding his way in this maze, fell back upon older and simpler systems, and to this day has scarcely recognized the "system of Eichler". Gray's treatment, however, was widely copied. The evolution of his textbooks was closely paralleled by that of Wood's "Classbook" (114), in which the scorpioid cyme appeared first in 1861. It is interesting that Bastin's figure 164 (6) clearly suggests that the "umbel" of *Allium* is not what the definition calls for. Crozier's "Dictionary" of 1892 (25) more nearly approached the European understanding of inflorescence.

CYMOSE AND RACEMOSE

It has occasionally puzzled students that flower-clusters should be universally classed in two main groups which are extremely difficult to define. If one can not accurately point out the distinction, of what use are they? The foregoing review has revealed that the classification is less a matter of scientific convenience than one of the historical development of ideas (much like the division of chemistry into its "organic" and "inorganic" realms). We use such terms as "cymose" and "racemose" mostly because of some now wholly outworn theories of Linnaeus, Goethe and Link. With a greater wealth of data and with a more acute analysis it became apparent to many botanists of the past century that the two groups of inflorescences cannot in fact be separated by any recognizable and clearly stated principle.

These difficulties, noticed by Hofmeister, Eichler and others, led first to a variety of attempts to define cymose and racemose by new criteria; these were reviewed by Čelakovský (21) and again by Goebel (36, p. 81 *et seq.*). The presence of a terminal flower is unsound as a criterion; one has only to recall *Campanula, Rubus* and *Berberis.* "Nicht alle Blütenstände, die eine Endblüte haben, können als cymös bezeichnet werden!" (36, p. 83). To characterize the cyme by the greater degree of branching of the lateral branches over that of the main axis, as the authors of the "Bonn textbook" (102) did, is to make many a compound umbel a cyme—a reductio ad absurdum. Nägeli in 1883 (82) varied this attempted solution by introducing the terms "untergipflig" and "übergipflig", according as the main axis or the lateral branches, respectively, are best developed. Again the "condensed racemes" offer difficulties. As for the order of flowering, if we disregard the axioms of Link and his followers, this is not a morphological character at all. Various authors have called attention to its variability. Obviously again *Rubus,* whose terminal flower may open first and be then followed by the flower most distant from it, conforms to neither of the commonly described patterns. In recent papers I have called attention to the futility of attempting to use the order of flowering as a morphological character in interpreting certain flower-clusters (90, 91).

Čelakovský (21) attempted to alleviate the difficulty by adopting a tripartite classification into paniculate (rispige), botryose (botrytische) and brachial (a substitute for cymose), as originally suggested by Wydler (118). The distinction is interesting. The number of "coordinate branches" (branches of the main axis) is compared with the number of sets of "subordinate branches" (branches arising from branches). In panicles both numbers may be unlimited. In "Botryen" the first is high, the second limited to two. In "Brachien" the second may be high, the first limited to two. The expression of this difference as an algebraic formula seems to have little significance, since there are no calculations to be performed. Čelakovský, like so many others, was drawn into the vortex of classification of "compound" types, and much of his effort went to a rather sterile discussion of the classification of "Trugdoldenrispe" (false-umbellate panicles) and the like. The term "thyrsus" is applied to the entire paniculate group. In a later work (22) the same author showed the close connection between brachial and botryose flowering in an elucidation of the heads of the Dipsacaceae.

To the extent that inflorescences can actually be classified in two groups (and such a classification is of dubious usefulness), the only sound basis has been expounded by Goebel in the masterly review already cited (36). In a noteworthy chapter on the Campanulaceae, in which family a great variety of inflorescences may be found, he shows (see especially figures 54 and 72 and relevant text) how easy it is to pass from "racemose" to "cymose" or the reverse. All that is necessary for the former change is the presence of a terminal flower, and the failure of flower-buds to develop from the lower axils, which confines their appearance to the axils immediately below the terminal flower. The reverse change occurs when the lower axils predominate in development. The former condition is spoken of by Goebel as acrotonic (akroton), the latter as basitonic (basiton). This is the distinction towards which Hofmeister, Nägeli and the authors of the Bonn textbook were striving.

Goebel's discussion focuses attention upon what has been throughout the greatest difficulty with concepts of inflorescence. In the hands of the writers of textbooks and descriptive manuals inflorescence has become a static thing—almost a dead thing. Linnaeus was perhaps on a better track when he thought of inflorescence not as a structural part of the plant but as a modus florendi. If his successors had studied modes of flowering rather than limiting themselves to attempting to name the finished products, the results might have been more applicable to living plants. It is true that Link and Roeper apparently were doing just that when they formulated a system on the order in which flowers develop. But the order of flowering is, as various workers have noticed, the least reliable aspect of the development of an inflorescence; and their principles were, as I have already shown, based upon *a priori* axioms and upon Goethean metamorphosis rather than upon a study of the living and growing plant. The dangers of attempting a discussion of inflorescences purely as matured structures appears from a consideration of theories of their origins, phylogeny and modifications.

In trying to put the study of inflorescence upon a modern basis, we encounter the same lack of morphological study which exercised Schleiden a hundred years ago. Figures of sections through young inflorescences may be found in the works of numerous authors, particularly Goebel (35, 36); they often show terminal flowers and lateral flowers in various relative sizes and stages of development. But, irrespective of which develops most rapidly and opens earliest, which was initiated first? One is tempted, in the absence of detailed study, to make the most natural assumption: that an axillary meristem, which may become a flower, is a residue of the terminal meristem which formerly occupied that level, and therefore antedates the existing lateral and apical meristems at higher levels.

The difficulties in the way of interpretation of inflorescence are well illustrated by the work of Müller (80) and others (18, 19, 81, 97, 99) who studied the development of the cincinni of Boraginaceae. The developmental history revealed by microscopic study may not correspond to the phylogenetic changes in the structure. The cincinnus may develop in the same manner as a simple raceme, though the mature inflorescence bears unmistakable evidence of its sympodial origin.

If the distinction between cymose and racemose is untenable, that between simple and compound inflorescences is no less so. Attention has been several times directed to the fact that when one speaks of an inflorescence as centrifugal one is comparing branches of different orders; or, to put the same facts in a different light, a simple cyme normally consists of not more than three flowers. This is the dichasium of Schimper in its most elementary stage: a terminal flower with two lateral flowers immediately below it and subtended by its prophylls. If the dichasium becomes once compound—by the branching of the lateral branches—then it is no longer centrifugal in a simple sense. It now consists of the original terminal flower and two lateral groups of three flowers each, in each of which the central flower may open first. As the dichasium becomes more ample, the same principle applies. Similar remarks may be made of monochasia and pleiochasia.

Only rarely are clusters encountered of more than one flower in which the terminal blooms first and the order is simply basipetal. Such a cluster is occasionally seen in some species of *Crataegus* (90), where it is apparently a secondary condition; the pedicels of the individual flowers are vestiges of more complex clusters, several of which were aggregated along a common axis. This apparently simple cyme is therefore really a compound inflorescence, and can not be understood without recognition of that fact.

It is clear also that the "capitate" clusters of *Cornus canadensis, C. florida* and other species of this genus are descended from widely branched dichasial panicles, from which the more open cymes of *C. racemosa* and *C. stolonifera* also have been derived. The aments of Juglandaceae, Betulaceae, the spikes of *Typha,* are known to have been derived from compound clusters (1, 20, 73), as the spikes of grasses and of Polygonaceae are anything but the simple structures called for by the definition of this term in the manuals. Likewise the umbels of *Allium* and other liliaceous genera are quite certainly derived from compound clusters, probably from bostryches resembling that of *Hemerocallis,* described in detail by the Bravais'. Considering the increasing multiplicity of such facts, it seems idle to insist on defining spike or raceme as simple inflorescences (containing only branches of the first order), since to do so is to remove from such categories most of the species commonly adduced as exemplifying them. In short, to make a primary division of inflorescences into simple and compound (save perhaps for an artificial key of limited application) is to sever at the outset the main arteries through which the understanding of inflorescence can be nourished.

THE PHYLOGENY OF INFLORESCENCES

Perhaps it is idle even to speculate on the origins of inflorescences, since we know so little of the relationships of the families of flowering plants, and since by reduction in number of flowers and condensation of branches the same patterns may be attained from different beginnings. However, merely to present for examination some of the possible answers will further illustrate the inadequacies and the inherent confusion of present practices and perhaps suggest a more rational approach.

The most usual assumption is that made by the fathers of botany and expounded by de Candolle in the "Organographie": that flowers are axillary branches. Flowers arise from buds; buds arise in the axils of leaves; ergo flowers originate in the axils of leaves. Their presence causes the modification of the subtending leaves. which become bracts. If the pedicels develop prophylls, this is a further illustration of their homology with axillary branches. As flowers arise from the axils, the terminal meristem of the main axis continues to develop in the usual way, until it is finally used up, sometimes by the end of the growing season, sometimes by the lack of available nutrient, sometimes by the formation of a terminal flower.

This account has the merits and the dangers of simplicity. Four considerations may be advanced: (*a*) As has already been noticed, inflorescences are rarely as simple as they seem. Spikes and heads are frequently derived from complex branch-systems; it is probable that most racemes also are, and that the apparently primitive axillary flower-bud may be a remnant of a more elaborate system. (*b*) The frequency of several-flowered clusters, which may be cymose, in the lower axils of a racemose system (*e.g.,* in *Campanula rotundifolia*) should arouse suspicion of the primary nature of the racemose system. (*c*) It seems to have escaped the notice of several generations of botanists that the assumption of two contrasting systems of inflorescence is gratuitous (*cf.* Parkin in 1914). Are flowers sometimes terminal structures, other times axillary? We have a right to expect a statement of the fundamental nature of inflorescence which should underlie any classification. (*d*) The supposedly primitive racemose condition is found in rather specialized groups: *Hyacinthus, Delphinium,* Leguminosae, Cruciferae, Ericaceae, Orchidaceae. It is more rarely encountered in more generalized or supposedly ancient groups as the Magnoliaceae, Rosaceae, Malaceae, *Ranunculus, Anemone, Clematis,* and many others. Arber (2) pointed out the dangers of oversimplifying the relationships of the racemose types; but his argument is weakened by the introduction of teleological factors.

In contrast with the customary view just outlined, a number of writers have proposed that the primitive inflorescence consisted of terminal flowers. Saint-Hilaire's idea has already been noted. Nägeli in 1883 (82) showed how both racemose and cymose types could be derived from a panicle by "übergipflig" or "untergipflig" tendencies. He was perhaps the first to emphasize the part played by reduction in phylogeny; a part now more generally realized. A similar concept was elaborated by Čelakovský (21) who presented the panicle as the most generalized inflorescence, from which racemose and cymose types may be easily derived by reduction. In 1914 Parkin (85) suggested that the most primitive condition was the single terminal flower. His figures show how from such a beginning could be derived both cymose and racemose types of clusters. Axillary flowers are obtained by shortening the flower-bearing branch into a pedicel. A number of branches with terminal flowers can thus yield a raceme. Or, if only branches near the end of a main axis are involved, the result may be a cyme. In Parkin's view, the simplest inflorescence is a dichasium: a terminal flower with two lateral flowers just beneath it. From such a type he derived more ample clusters by the production of additional branches in the axils of the leaves of the lateral branches. "Another point . . . requiring some clearness of thought, is the recognition of an essential difference between floral and vegetative branching."

The latter he considered to be monopodial in origin, sympodial only as a secondary development; while floral branching is necessarily sympodial, since a flower terminates the growth of every flowering axis.

In 1922 Pilger (87), commenting that the herbaceous dicots, including such forms as *Paeonia* and *Helleborus* on which Parkin based much of his argument, are secondary and do not represent primitive groups, proposed a modification of the theory that inflorescences are derived from terminal flowers. The simplest situation, he said, would be a system of small twigs, crowded together, each terminated by a flower. This he supports by a dubious analogy with *Chamaecyparis,* and then admits that such an arrangement is never found in living woody angiosperms.[17] From this type of flowering—the beblätterte Rispe—Pilger would derive a panicle consisting of numerous leafless branches closely crowded and bearing terminal dichasial groups of flowers. Such a panicle by reduction in either its terminal or its lateral portions, or in both, could yield various types of thyrse, axillary clusters, a simple terminal dichasium, or a solitary terminal flower.

The two theories just outlined both depend on leafy twigs terminated by flowers. The first, Parkin's, supposes that an inflorescence can begin with one such branch, later branches being added by axillary growth. The second, Pilger's, begins with a large number of branches which remain simple but are condensed into a system by the disappearance of intervening leaves and the shortening of internodes. It is evident that the second theory has the best of it. Parkin's theory is really self-contradictory, in beginning with a terminal flower and then calling for help from the axils. If axillary growth can indeed yield branches terminated by flowers, it is far easier to suppose that such growth occurred from the beginning rather than waiting its turn in the development of a compound inflorescence from a single flower.

Zimmerman (120) also takes as his starting-point the "cymose panicle" (zymöse Rispe).[18] He also admits that this type of cluster

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[17] "Der einfachste Fall wäre der, dass an einem reicher System von gedrängten kurzen Zweigen alle Zweige nach Hervorbringung von Laubblättern eine Terminalblüte erzeugen. Dieser Fall, der dem eigentlichen Blütenstand vorausginge und dem *Chamaecyparis*–Typus entsprache, existiert bei den heutigen angiospermen Holzpflanzen nicht".

[18] "Zymöse Rispe nenne ich . . . einen Blütenstand, der sich wie allen Rispen wiederholt verzweigt, und der (als 'zymöses' Blütenstand) die Terminalblüten sowohl am Gesamtsystem wie an den Seitenzweigen jeweils vor den darunter inserierten Blüten anlegt und entfaltet".

"in ganz typischer Form" is rarely found. The "leichte Modificationen" he imagines to have occurred are differentiation of bracts from leaves, development of dichasia at the tips of branches, and occurrence of early-blooming flowers on the lower side-branches. Since these "small changes" entirely transform the inflorescence and evade the whole morphological issue, it does not seem profitable to attempt to discuss his theory further. Such a decision is confirmed by the discovery that to this writer the inflorescence of *Daucus carota* is a "zymöse Rispe"—the entire shoot is inflorescence, consisting of several compound umbels arranged in somewhat corymbose fashion. He postulates a number of "primitive characters" obviously derived from consideration of leaf-bearing shoots. To support his insistence upon the importance of the order of flowering (although this is so easily modified), Zimmerman adduces the classic theory of the origin of the flower by aggregation of "sporophylls" at the tip of an axis, and by several *a priori* considerations of the necessity of the "voraneilander Terminalblüte".

In a careful paper on the inflorescence of Apocynaceae, Woodson (115) remarks that "the origin of the inflorescence is at least as remote as the origin of the flower". This thought, which the author did not further develop, is a ray of light in the prevailing murk. We are not yet ready to make any very decisive pronouncements on the phylogeny of the flower; but few will hesitate to admit that a flower is a branch; and evidence is accumulating (42, 103, 110, 111) that it is derived, at least in part, by condensation from a system of branches. Individual flower-parts are not leaves now and probably never were.

Let us go a step farther. If it is no longer necessary to found our phylogenetic systems on leaves and nothing but leaves, we may recognize the possibility that the origin and development of the flower was coeval with that of the angiosperm leaf, not subsequent to it. It is worth while to recall here that one current theory of the origin of leaves themselves is that they are condensed and originally dichotomous branch-systems (26). It is possible that the branches which coalesced to form a flower were homologous with those other branches which were at the same time giving rise to leaves. This is not the place to debate such theories; nor, perhaps, does the present state of our knowledge warrant an extended discussion of them. They are here adduced only to show what vistas are possible once we have freed ourselves from the dead hand of Goethe.

Such a liberation of thought is particularly desirable for the consideration of inflorescence. If we think of the ancestors of flowers as forming a group of reproductive branches, such a system was in effect an inflorescence even before the first flowers had taken on something like their modern form. The inflorescence may be older than the flower. If so, it is obviously idle to attempt to derive inflorescences by homology with vegetative branches—as idle as it is to attempt to understand a flower by homologizing its parts with foliage.[19]

Such considerations throw new light on the various proposed theories of the phylogeny of inflorescence. Let us grant, first, that a flower is indeed a terminal structure; it does not merely seem to be so, but is—Roeper and teratological manifestations to the contrary. Even an "axillary" flower is terminal upon its pedicel, as Turpin said in 1819; the "sessile" flower is a morphological myth. Whatever its phylogenetic origin, a flower arises from an apical meristem. It is perhaps significant that all modern writers on inflorescence have been led to regard the terminal position as primitive. There seems no reason, however, for limiting the primitive inflorescence to a single terminal flower, as Parkin did, nor for beginning with Pilger's "leafy panicle". Both these efforts are weakened by the effort to homologize vegetative and reproductive branches; both started with foliage, which then has to be got rid of. Let us first eliminate from our minds the necessity of foliage in the primitive inflorescence. Bracts there may have been from the beginning, as a development parallel with that of leaves elsewhere on the plant.

Granting some kind of primitive leafless panicle, it is easy to derive from it all manner of inflorescences, with and without foliaceous bracts. Woodson in the paper just cited has a number of diagrams which do this for the inflorescences of Apocynaceae, and the same story is worked out by Goebel in his chapter on Campanulaceae (36). In spite of the vagueness of our concepts of the origins of flowers, we may—at least as a working hypothesis—

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[19] The temptation towards such homologies, strengthened by long habit, is insidious. Woodson, author of the illuminating statement already quoted, on another page says "the inflorescence is essentially a phase of branching of the shoot system but it is vastly more complicated than vegetative branching, from which it has been derived".

adopt the view that the primitive inflorescence consisted of flowers borne terminally on a system of branches. Such a doctrine would not exclude the possibility that development had occasionally occurred in the opposite direction—from axillary to terminal—as Goebel maintained was true of the grasses (35, 36).

Since it may be considered unsporting to knock down the theories of others without setting up something for them in turn to shoot at, the following is presented as a basis for the phylogeny of inflorescence. In dealing with complex clusters it is noticeable how often one encounters a dichasial group of flowers as a sort of unit cluster, the entire inflorescence being composed of many such units variously related. I have recently advanced this interpretation of the inflorescences of *Crataegus* and *Philadelphus* (90, 91). Woodson took a dichasium as the starting point for the inflorescences of Apocynaceae. Parkin supposed the dichasium to be the simplest inflorescence. The complex clusters of *Cornus* may be similarly analyzed; also the ample thyrse of *Syringa*.[20] A number of families—Crassulaceae, Caryophyllaceae are the most conspicuous examples—display inflorescences obviously dichasial. And of course the common monochasial systems—*e.g.*, in *Hemerocallis, Allium, Claytonia, Labiatae*—are easily derivable from a dichasium and, in fact, often begin in one.

A dichasium—I use the word in its strict sense to mean a cluster formed by an apparent dichotomy beneath a terminal flower—a dichasium in its simplest form consists of three flowers. This is essentially the "archibrachium" of Čelakovský (21), who also saw in it a primary form. It may originate very simply by two rapidly successive divisions of the apical meristem (perhaps indeed dichotomies); the same sort of branching which is frequently encountered among cryptogams and which is invoked to explain the origin of a monopodially divided leaf from a dichotomously forking shoot. The simple dichasium has the earmarks of a quite primitive type of organization; future investigations of the inflorescence may well adopt it as a starting point. The assumption that this structure is indeed primitive has the further advantage that it is derived not from any set of rules about what should be considered primitive in a branch, nor from any presuppositions about the arrangement of "sporophylls", but purely from observation of modern inflorescences and an attempt to analyze the structure of the more complex types.

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[20] Woodson rather thoughtlessly said that "in the true panicle, as represented by the common lilac, *Syringa vulgaris* L., the subsidiary axes, whatever their number, are likewise indeterminate" (115, p. 6). Whatever be the proper definition of a "true panicle"—and of this more later—it is easily verified by ordinary observation that the ultimate branches of a lilac thyrse are "determinate".

From a simple dichasium ("simple cyme" in the terminology current among some of my friends) a more ample system may be obtained by repetition of the branching. That is, the lateral branches of the first tripartite cluster can repeat the process instead of maturing at once into flowers. The compound dichasium may be the "Rispe" demanded by Pilger and Zimmerman, and may extend very far back in angiosperm history. In preceding it by the simple dichasium in this discussion I do not imply that it was necessarily later in evolutionary history. The first inflorescence may have already been a much-branched system; the transition is equally easy in either direction. In each ultimate dichasial cluster the terminal flower usually opens first; but not necessarily. This is rather a physiological than a morphological matter, as is probably the well known separation of the sexes in *Begonia* (75). From a primitive dichasium may be derived also, without extensive changes, bostryches, cincinni, and the rest; and by a shortening of internodes many of the "umbellate" and "capitate" clusters characteristic of liliaceous and cornaceous genera.

It is otherwise with the formation of various more congested flower-clusters. The foregoing paragraphs must not be read as proposing that *all* modern inflorescences have originated solely from leafless reproductive branches—an obvious absurdity. If we grant the existence of a primitive type of reproductive branch-system, dichasial in structure, it is certain that the clusters originally representing the primitive type have been combined and aggregated in a variety of ways, with reduction and loss of intervening foliage, to form what Goebel (36) has termed a synflorescence (Synfloreszenz). In fact the "racemose" types of inflorescence, which are probably compound in their origin, must have arisen by such a process. The steps in such an evolution are (*a*) limitation of the individual dichasia to a few flowers, often two or one; (*b*) grouping of branches bearing leaves and terminated by dichasia on a common axis, a grouping which involves a shortening of the branches and of the internodes between them; and (*c*) reduction of leaves to bracts. A typical example may be seen by comparing several species of *Philadelphus* (91). Another familiar example of the development of the "indeterminate" inflorescence is found in the Labiatae. The axillary "whorls" or "verticels" of small flowers so common in this family have long been recognized (*cf.* de Candolle in 1827) as being "cymose"—usually monochasial groups of some kind. As each such group approaches reduction to a single axillary flower, the subtending leaves become changed in character and the internodes between them shortened; from many "determinate" inflorescences we get an "indeterminate" raceme or spike.[21] There can be no doubt that reduction of foliage leaves has played a part in the development of inflorescences; but there is no consequent necessity for clothing with foliage the ancestral dichasia and monochasia.

One consequence of the adoption of any form of terminal flowering as primitive remains to be investigated. The so-called prophylls are the first leaves (or leaf) of a lateral branch. In the effort to homologize flowers with lateral branches, the term was carried over to apply to the bracts (bracteoles) immediately beneath the flowers. The prophylls have been described as bracts on the pedicel, as distinguished from the bracts (Tragblätter) which subtend the pedicels. In a raceme this homology may indeed be valid. The flower-stalk may be all that is left of a lateral branch, and the prophylls of the latter may become the bracteoles of the former. But the nature of the bracteoles of a dichasial cluster is not so clear. The flower which terminates the leafy axis should have no prophylls; yet it may have bracts which exactly resemble those on the lateral flower-stalks. If the word prophyll is used in connection with flowers, it should be divorced from any implied homology with leaves of vegetative branches until such time as flowers and flower-clusters are more thoroughly investigated. Certainly the production of one or more bracteoles seems closely related to flower-formation, so much so that they may almost be regarded as parts of the flower. Even when they are lacking, there is frequently a joint in the pedicel indicating some sort of morphological transition.

At this point it is worth noting that with all the impressive vocabulary developed in textbooks and manuals to deal with inflorescence, some common types have received no name and, in fact, no recognition save by a few morphologists. The term cladanthy (Kladanthie) proposed by Winkler (112), while it does not refer to a true flower-cluster, may be useful to designate the production of flowers irregularly on the internodes of a vegetative branch. Another useful concept is the intercalary inflorescence described by Parkin (85). This results from a condensation of separate clusters upon a central axis which may remain vegetative at its tip. The resulting system consists of a branch which passes from a vegetative to a reproductive phase and then back to a vegetative.[22] Another even less recognized system—scarcely an inflorescence—is what Goebel (36) has termed the "anthoclad" (anthokladium). This is a system which constantly wavers between vegetative and reproductive development, producing at the same time flowers or flower-clusters and branches which bear foliage. The system may be recognized for what it is by the fact that the branches are rarely typical of the vegetation of the plant below the onset of the reproductive phase; they differ commonly in the arrangement and form of their leaves. Examples of this development are numerous and familiar—Geraniaceae, including the household *Pelargonium,* Solanaceae, Asclepiadaceae, Euphorbiaceae, among many others. In common Euphorbiaceae the visible plant body is largely anthoclad, *i.e.,* neither vegetative shoot nor reproductive cluster but both in a special combination. For an elucidation of these interesting relationships the reader should consult Goebel's exposition of inflorescences, already several times cited (36).

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[21] We may note in passing a paper by Urban (107) on the biological advantages of the aggregation of inflorescences, with interesting notes on the manner of such aggregation.

CONCLUSIONS

What, if any, should be the effect of the foregoing considerations upon our treatment of inflorescence—particularly upon the descriptive terminology of inflorescence?

It is evident, first, that we need a terminology which will work. It is of no use to define a set of terms so as to exclude the plants to which we should like to apply them. If morphology demonstrates that the spikes of common plants are not what is so defined in the manuals, then the definition must be changed. Enough has been said to show that our current definitions are out of date and inadequate for their task.

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[22] Before this paper appears in print an article by Dr. Leon Croizat will be published in the Bulletin of the Torrey Botanical Club. In this paper, which I have had the privilege of reading in manuscript, Dr. Croizat calls attention to the strange neglect of the intercalary inflorescence, and adduces some examples. It is a pleasure to acknowledge here my debt to this writer, to the many stimulating conversations in which we have exchanged views. Most of the present discussion, however, was written before I saw the manuscript referred to, and my conclusions, while in part identical with Dr. Croizat's, were reached independently; the same, naturally, is true of his.

Second, our terminology should be based on fact, not fancy. Flowers have been classified by the emotions with which they have been associated; but it is difficult to recognize any scientific value in the "language of flowers". It is not much more fruitful to classify inflorescences in the light of a physiology and a philosophy which have not been seriously regarded for a century.

Fortunately the type which I have taken as primitive, and which is certainly common, the dichasium, is well founded and easily defined. It may be simple or compound, ample or restricted; no great exercise of the imagination is needed to formulate the necessary descriptions. It should be pointed out that the word will retain its usefulness only if used in its original sense: a dichasium originates in a single peduncle by "dichotomous" branching immediately beneath a terminal flower, and develops by a repetition of the same apparent dichotomy in each lateral branch. Nor need we fear any great difficulty in naming the familiar types derived from the dichasium by suppression of flowers in recurrent positions: bostryx and cincinnus, drepanium and rhipidium. A little more care must be exercised by the systematist than he has been accustomed to use in describing inflorescences; this is inevitable in the development of his science. It is essential first to eschew the carelessness which has confounded different monochasial clusters under one name ("scorpioid cyme") and to rewrite many of the definitions in the manuals. It should be noted also that the inflorescences of this important group are "compound" in the older sense even when they appear simple (*e.g.*, the apparent raceme of *Claytonia*), and cannot be characterized accurately as determinate or indeterminate, centrifugal or centripetal. In a dichasium each ultimate cluster of three may perhaps be centrifugal, though not necessarily so. In a monochasium the development of a sympodial "false axis" may result in an apparently centripetal inflorescence, which, however, is really built of determinate units. If it is desirable to characterize the entire group of inflorescences by a short phrase, the term "sympodial" comes to mind.

Certain inflorescences derived from extreme condensation of a sympodial cluster may closely simulate umbels which have a monopodial origin. Since our terminology is to be used by systematists, who cannot always postpone their conclusions for microscopic investigation, it would be wise to use the term "umbel" for all clusters whose branches arise from a common point. Enough has been said to demonstrate the fallacy of incorporating the order of flowering into the definition; in fact, though apparently such an easy criterion, it has not worked well, as witness *Pelargonium* and *Asclepias*. When the morphological nature of an umbel (in the above sense) is understood, it may be designated as sympodial or monopodial. Such a procedure, which makes use of existing concepts and terms so far as they are tenable, is preferable to coining a new terminology. As Hy wrote in 1894, "Il convient de modifier et d'améliorer un langage defectueux, plutôt que tenter un changement radical . . . ."

The status of the dichasium and monochasium is clear; that of the pleiochasium is less so. There is no *a priori* reason why a primitive and still widespread flower-cluster should be limited to three primary branches; but it remains an observed fact. In introducing the term "pleiochasium" in 1875, Eichler wrote that it was of small morphological interest and of restricted occurrence (29). As he further noted, the pleiochasial branching is not usually maintained in subsequent development, but goes over into a di- or monochasial system. Moreover, if the branches are numerous, the pleiochasium approaches a botryose cluster; and in fact Parkin identified panicle and pleiochasium. My suspicion is that the supposed pleiochasium is usually a secondary grouping, as in *Crataegus pruinosa* (90), caused by condensation of several originally dichasial branches upon a common axis; in short, a synflorescence. If this is correct, the term will tend to disappear as the true nature of particular clusters becomes understood.

When we have disposed of the dichasium and its immediate relatives we have broken the back of our problem; for these "cymose" clusters, as the Bravais' realized and current textbooks do not, are very numerous. We have remaining the numerous forms of the "racemose" or "botryose" types, and the frequent more ample inflorescences generally known as compound—panicles, thyrses, and the like.

The terminology of the racemose group of inflorescences is already in a fairly satisfactory state, even if the terms are quite often misapplied. There is no need here to elaborate the definitions of raceme, spike, corymb, umbel, capitule, which are usually clear enough in current treatments. Attention has been already directed to the necessity for care in the recognition of these types and to the desirability of qualifying them so as to distinguish the monopodial varieties from the sympodial clusters which they may closely resemble.

The cincinnus or bostryx may closely simulate a raceme (*e.g.*, in *Claytonia*), and those who place too much reliance in acropetal flowering may be misled; but careful examination will reveal that each bract subtends the next joint of the apparent axis rather than the pedicel of a flower. There is, however, no innate necessity for limiting racemes and corymbs to "simple" clusters, especially if my contention is upheld that they are all compound in origin. The branched racemes of *Veratrum* and *Smilacina* are better described as such than by the protean term "panicle".

In coming to grips with larger and obviously compound clusters of flowers, such as those of *Viburnum* or *Syringa,* the same principles should be our guide. Various ingenious botanists have manufactured elaborate systems of terms (12, 22, 29, 44) based on a correspondingly involved classification of such clusters. Such schemes take off from the old bifurcation into "determinate" and "indeterminate" inflorescences. One may recall de Candolle's idea of thyrse and corymb, and the Bravais' sarmentide, as examples. Furthermore, they all imply that the inflorescence is "built up" out of this or that kind of unit—instead of being derived by the coalescence of originally separate clusters. Such terminologies are therefore fundamentally fallacious as well as a nuisance to use; it is significant that they have had few takers.

As I have noted above, a striking feature of many such flower-clusters is the fact that the ultimate units are dichasial or show traces of having once been dichasial. These groups, often reduced to single flowers and denuded of any bracts that may have been present, become closely aggregated on a common axis without any discernible order, certainly without any evident "racemose" or "cymose" scheme of flowering. Such axes are themselves variously disposed on larger branches, and so on. The primary branches may retain something of the arrangement and developmental relationships which characterized them before condensation and reduction set in. They may even mature their flower-clusters more or less acropetally, though no such progression may be found in each cluster; but this is apparently not very common and it is rarely possible to make any exact statement about the order in which flowers of such compound clusters open. Two well established terms may be of use here, though their adoption may necessitate a certain reorientation in the minds of those who use them. The term "cyme" was long used for flat-topped or convex compound clusters of the type exemplified by *Sambucus, Cornus, Viburnum* and *Crataegus,* and this usage has occasionally persisted to the present day, even though the definition of cyme has been changed. I have already proposed (90) that the word be redefined so as to apply to these more or less flat clusters composed of essentially dichasial elements. It should be unnecessary at this point to dilate further on the lack of significance of order of flowering in any attempt to name such clusters, and the definition of cyme will benefit by the elimination from it of any such qualifications.

The thyrse offers less difficulty, being already tolerably well explained in many treatments. It is essentially like the cyme (as defined above) but of cylindrical or pyramidal form. Again we should eschew all reference to order of flowering.

Finally, that much abused word "panicle" must be clarified, if it is to be used at all. Its original use was very vague; it answered for any loosely branched flower-cluster, as exemplified particularly by the grasses. By some early authors it was limited to grasses. Recent attempts to define it have sought to make it a compound raceme, an inflorescence in which the ultimate branches are racemose as well as racemosely arranged on the main axis. Such inflorescences, though uncommon, do exist, particularly among grasses; but meanwhile the term "panicle" has been habitually used for many inflorescences which cannot be so characterized. In order to retain some semblance of usefulness, the term must cast off such mistaken efforts at precision and return to its ancient paucity of conceptual raiment; it is simply a loosely branched system. Its ultimate units may be and frequently are dichasial like those of a cyme or thyrse; but I see no necessity for limiting the concept to a cluster so composed any more than to a wholly "indeterminate" system. The two subfamilies of grasses apparently differ in the nature of their spikelets;[23] no one will seriously propose to limit the term "panicle" to the Festucoideae.

In conclusion, let us make a gesture in the direction of the word "inflorescence" itself. I have already called attention to the change of meaning that overtook it early in the nineteenth century. As botanists became interested in naming structures rather than understanding their development, a new emphasis was inevitable; inflorescence became a structure rather than a modus florendi. This is perhaps unfortunate. While it is useful to have a term to describe the structure—and "flower-cluster" may seem to many rather vague and amateurish—we feel the lack of a means of referring to the concept of flower-arrangement, a concept which embodies certain principles. The attentive reader will have perhaps noticed that in an effort to satisfy both needs I have in this paper occasionally used "inflorescence" without an article in the more abstract sense. In this sense a study of inflorescence may involve a consideration of the origins and development of the modes of flowering. The insertion of the article or the use of the plural at once reestablishes the usage which has become so customary that it would be foolish to attempt to change it: an inflorescence is a flower-bearing branch or system of branches.

APPENDIX: AUTHORSHIP OF TERMS FOR INFLORESCENCES (SINCE 1751)

This list is offered for the benefit of those who like to trace the history of terms; to judge by my correspondence, they are not few. Although (I repeat) the application of technical terms is not to be settled by priority, there may sometimes be advantage in selecting from many meanings that of the first user, unless other considerations intrude. Moreover, as I have attempted to show, to trace the term to its beginnings is often to gain a better understanding of the relationships to which it refers. To associate a term with the name of its first user is to revert to a useful practice often followed by early botanists.

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[23] Even if this difference is more apparent than real, the working systematist must be guided to a certain extent by appearances. If panicles were strictly indeterminate, it would be difficult to include those of *Panicum* and other genera which have apparently terminal-flowered spikelets.

The list is necessarily incomplete, and it may be that some of the references cited are not the earliest. I shall be grateful for terms and references not here mentioned.

The name of the author follows the term which he introduced and is in turn followed by the date of publication; the first number in parentheses refers to the list of literature cited. When the word was introduced both in Latin and in a modern language, I have usually given only the former.

Akroton: Goebel 1931 (36, p. 80).
Amentum: Linnaeus 1751 (69, p. 52).
Amphant[h]ium: Link 1798 (65, p. 109).
Anthela: Meyer 1819 (76, p. 11).
Anthémie: Guillard 1857 (43, p. 29).
Anthèse: Guillard 1857 (43, p. 121).
Anthodium: Ehrhart 1788 (28, p. 64).
Anthoecium: Link 1809 (66, p. 53).
Anthokladium: Goebel 1931 (36, p. 2).
Ant[h]ostegium: Link 1798 (65, p. 79).
Anthotaxy: Gray 1879 (41, p. 141).
Anthurus: Link 1798 (65, p. 59).
Archibrachium: Čelakovský 1892 (21, p. 43).

Basiflora (inflorescentia): Link 1798 (65, p. 57).
Basiton: Goebel 1931 (36, p. 88).
Bipare (cime): Bravais & Bravais 1837 (12, p. 196).
Bostryx: Schimper ex Braun 1835 (11, p. 189).
Botry-cyme: Guillard 1857 (44, p. 375).
Botry(d)e: Guillard 1857 (43, p. 122).
Botrys: Čelakovský 1892 (21, p. 43, 45).
Brachium: Čelakovský 1892 (21, p. 42).

Calathidis: Mirbel 1815 (79, p. 283, 778).
Calathidium: Link 1824 (67, p. 258).
Calopodium: Nees von Esenbeck 1821 (83, p. 24).
Capitulum: Linnaeus 1751 (69, p. 41).
Catulus: Berkenhout 1764 (8, sub amentum).
Centralis (inflorescentia): Link 1798 (65, p. 56).
Centrica (inflorescentia): Link 1798 (65, p. 56).
Centriflora (inflorescentia): Link 1798 (65, p. 56).
Cephalant[h]e: L. C. Richard 1798 (90, p. 183).
Cephalanthus: Mirbel 1802 (78, **2**: 202).
Cicinnus: Wydler 1851 (118, p. 307).
Cicinus: Schimper ex Braun 1835 (11, p. 189).
Cincinnus: Wydler 1851 (118, p. 307).
Clinanthium: Mirbel 1815 (79, p. 273, 752).
Coenanthium: Nees von Esenbeck 1821 (83, **2**: 51).
Corymbothyrsus: Čelakovský 1892 (21, p. 46).
Corymbus: Linnaeus 1751 (69, p. 41).
Cyma: Linnaeus 1751 (69, p. 55, 76, 78).
Cymo-botrye: Guillard 1857 (44, p. 375).

Definita (inflorescentia): Roeper 1826 (92, p. 442).
Determinata (prolepsis): Link 1824 (67, p. 246).
Dibotrye: Eichler 1875 (29, p. 41).
Dibrachium: Čelakovský 1892 (21, p. 43).
Dichasium: Schimper ex Braun 1835 (11, p. 189).
Dicyme: Eichler 1875 (29, p. 41).
Drepan[i]um: Buchenau 1866 (14, p. 393).

Eccentralis (inflorescentia): Link 1798 (65, p. 57).
Eccentrica (inflorescentia): Link 1798 (65, p. 57).

Fasciculus: Linnaeus 1751 (69, p. 41).

Glomerulus: Link 1798 (65, p. 59).

Helicoïde (cime): Bravais & Bravais 1837 (12, p. 197).
Heterogenea (inflorescentia): Link 1824 (67, p. 252).
Homogenea (inflorescentia): Link 1824 (67, p. 252).
Hypanthodium: Link 1824 (67, p. 265).

Indefinita (inflorescentia): Roeper 1826 (92, p. 442).
Indeterminata (inflorescentia): Roeper 1826 (92, p. 442).
Indeterminata (prolepsis): Link 1824 (67, p. 246).
Inflorescentia: Linnaeus 1751 (69, p. 112).
Intercalary (flower-cluster): Parkin 1914 (85, p. 512).
Involucrum: Linnaeus 1751 (69, p. 52).

Julus: Linnaeus 1751 (69, p. 113).

Kladanthie: Winkler 1931 (112, p. 96).

Mérithalle: Bravais & Bravais 1837 (12, p. 204).
Monobrachium: Čelakovský 1892 (21, p. 43).
Monochasium: Eichler 1875 (29, p. 34).
Multipare (cime): Bravais & Bravais 1837 (12, p. 196).

Nucamentum: Berkenhout 1764 (8, sub amentum).

Panicula: Linnaeus 1751 (69, p. 41).
Peranthodium: Link 1798 (65, p. 81).
Periphoranthe: L. C. Richard 1798 (89, p. 184).
Phoranthe: L. C. Richard 1798 (89, p. 185).
Phoranthium: A. P. de Candolle 1813 (16, p. 356).
Pleiochasium: Eichler 1875 (29, p. 34).
Prophyllum: Schimper ex Wydler 1851 (118, p. 292).
Pseudothalle: Bravais & Bravais 1837 (12, p. 197).

Racemus: Linnaeus 1751 (69, p. 41).
Rhipidium: Eichler 1875 (29, p. 35).
Rhipis: Buchenau 1866 (14, p. 392).

Sarmentide: Bravais & Bravais 1837 (12, p. 197).
Scorpioïde (cime): A. P. de Candolle 1827 (17, 2: 415).
Sertulati (flores): A. Richard 1819 (88, p. 156).
Sertulum: L. C. Richard 1798 (89, p. 133).
Spadix: Linnaeus 1751 (69, p. 55, 77).
Spatha: Linnaeus 1751 (69, p. 52).
Spica: Linnaeus 1751 (69, p. 41).
Spicula: Linnaeus 1751 (69, p. 223).
Stachyodes (inflorescentia): Link 1798 (65, p. 59).
Strobilus: Linnaeus 1751 (69, p. 53).
Sympodium: Schimper ex Wydler 1851 (118, p. 309).
Synfloreszenz: Goebel 1931 (36, p. 2).

Thyrsoid: Čelakovský 1892 (21, p. 46).
Thyrsus: Linnaeus 1751 (69, p. 41).

Umbella: Linnaeus 1751 (69, p. 54, 76, 78).
Umbellula: Linnaeus 1751 (69, p. 79).
Unipare (cime): Bravais & Bravais 1837 (12, p. 196).

Verticillaster: Bentham 1836 (7, p. xvi).
Verticillus: Linnaeus 1751 (69, p. 41).

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PRESENT-DAY CLASSIFICATION OF ALGAE[1]

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INTRODUCTION

During the latter part of the nineteenth century it was customary to recognise four main classes of algae (32, 52, 54, 152, 154): Chlorophyceae, Phaeophyceae (Melanophyceae), Rhodophyceae, Cyanophyceae (Schizophyceae, Myxophyceae). Diatoms and Peridinieae, likewise accepted as algae, were sometimes treated as separate groups (32, 139, 157) and sometimes classed among the Phaeophyceae (152, 154). Various authors (152, 154) also included in the latter a number of the Flagellata, now largely comprised in the Chrysomonadineae, while in the first edition of the "Natürliche Pflanzenfamilien" (128) the Flagellata as a whole were given a brief though very useful consideration within the compass of the Thallophyta. Most writers, however, altogether ignored the bulk of the Flagellata. This was an altogether illogical attitude, so far as the holophytic members of this series were concerned, for even at this period *Chlamydomonas* and other green starch-producing Flagellata (*Pandorina, Eudorina*) were included among the Chlorophyceae.

The modern era in algal classification commences with the removal by Luther (86) in 1899 of certain genera, hitherto referred to Chlorophyceae, to a separate class, the Heterokontae, distinguished *inter alia* by yellow-green chloroplasts, formation of oil as a photosynthetic product, and two unequal anterior flagella on the motile stages. The ground had been prepared by Borzi (16, *199*) and Bohlin (12) who had grouped some of the genera (*e.g., Botrydiopsis, Ophiocytium, Conferva = Tribonema,*) as Confervales, but it was the discovery of a flagellate, *Chloramoeba* (13), and of a palmelloid type, *Chlorosaccus* (86) showing heterokontan characteristics, that betrayed the existence of a larger entity than could be accommodated in a mere subdivision of the Chlorophyceae. Recognition of the taxonomic differences between the latter and the Heterokontae, as well as of the fact that each of these classes was distinguished by a definite type of flagellate organism, led to the adoption of a new attitude towards the heterogeneous group of motile forms known to zoologists as Flagellata. The new outlook found a fuller expression in a later paper by Bohlin (14) and, for English readers, in F. F. Blackman's (6) article on "The primitive algae and the Flagellata", while Blackman and Tansley's "Classification of the Green Algae" (7) was an exposition of the new point of view so far as the green and yellow-green algae were concerned. In a reprint published the following year (8) the designation "Isokontae" was adopted for the Chlorophyceae and remained in vogue for a considerable time, although more recent writers have reverted to the old name, and "Isokontae" has fallen into disuse. The desire for a uniform terminology for the various algal classes has also led in some quarters (41, 136) to acceptance of the name "Xanthophyceae" (3) for the Heterokontae, and this is followed in the present article.

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[1] In general only the broader aspects of classification of algae are taken into consideration, since a detailed discussion of the many problems relating to the placing of individual genera would unduly increase the length of this article.

INTERRELATIONS OF FLAGELLATA AND ALGAE

A further step leading to the present-day basis of classification of algae was afforded by an important memoir of Klebs (64) in which he described a number of coccoid types showing at certain stages of their development all the essential characteristics of the Peridinieae. It thus became apparent that, also in this group of dominant flagellate types, forms with an algal organisation had been evolved. It was left to Pascher, however, to carry to its logical conclusion the attack on the Flagellata initiated by the work of Bohlin, Luther and Klebs. In a striking paper published in 1914 (95) he showed not only that a close relation between flagellate and algal types was patent among Chlorophyceae, Xanthophyceae and Peridinieae, but that similar relations existed between the flagellate forms grouped by Senn (128) as Chrysomonadineae and Cryptomonadineae and corresponding algal forms briefly considered in Pascher's paper. The three classes with predominant flagellate organisation thus distinguished were designated Chrysophyceae (including the Chrysomonadineae), Dinophyceae (including the Peridinieae) and Cryptophyceae (including the Cryptomonadineae).

The flagellate members of each of these classes as well as of the Chlorophyceae and Xanthophyceae, possess distinctive accessory chromatophore pigments and produce different photosynthetic products, implying for each class a distinctive metabolism; they are also distinguished by their morphological features and especially by the nature and orientation of the flagella. Each class also includes a variety of palmelloid, coccoid and filamentous types, such as have long been known among the Chlorophyceae; among Xanthophyceae, Chrysophyceae and Dinophyceae their number has been appreciably increased by later communications of Pascher (98, 99, 102, 105, 107). Such algal forms show the physiological characteristics (pigmentation, photosynthetic products) distinctive of the flagellate types of their class, and many of them propagate by motile reproductive units which, also in their morphological characters, closely resemble the flagellate members. Pascher was the first to bring out clearly the essential parallelism between the different classes, a parallelism which is reflected in the broad classification given below and which, with some modifications, has been generally adopted. The flagellate, palmelloid, coccoid, filamentous and siphoneous types were relegated to separate orders, as indicated in the following synopsis, taken from the 1914 paper and slightly modified in the light of subsequent knowledge:

	Chlorophyceae	Xanthophyceae (Heterokontae)	Chrysophyceae	Dinophyceae	Cryptophyceae[2]
Flagellate	Volvocales	Heterochloridales	Chrysomonadales	Dinoflagellata	Cryptomonadales
Palmelloid	Tetrasporales	Heterocapsales	Chrysocapsales	Dinocapsales	Phaeocapsales
Coccoid	Protococcales (Chlorococcales)	Heterococcales	Chrysosphaerales	Dinococcales	Cryptococcales
Filamentous	Ulotrichales	Heterotrichales	Chrysotrichales	Dinotrichales	....
Siphoneous	Siphonales	Heterosiphonales	....	....	....

Of the pigmented holophytic Flagellata distinguished by Senn (128) there remain only two series—the Chloromonadineae and

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[2] The Cryptotrichales distinguished in the 1914 paper subsequently proved to be members of Chrysophyceae.

Euglenineae—in which, so far as present knowledge goes, no algal types have been evolved. The few rather complex motile unicells (*e.g., Vacuolaria, Gonyostomum*) included in the Chloromonadineae appear to have no affinities with the other groups, and future investigations must establish their exact status. The holophytic Euglenineae (*Euglena, Trachelomonas, Phacus, etc.*) exhibit no characteristics that support their inclusion among the Protozoa, and the zoological terminology suggestive of a process of holozoic nutrition is quite unwarranted and deplorable. As in other flagellate groups (Volvocales, Chrysomonadales, 112), there has been considerable evolution of colourless types, but the majority of them (Astasiaceae) are pure saprophytes and the peculiar holozoic nutrition of Peranemaceae is clearly derivative. There can be no doubt that the Euglenineae are a group parallel to the flagellate series of the five algal classes referred to above, and future research may yet disclose true algal representatives of this class. Until these are discovered, use of the names "Euglenophyceae" (135) or "Euglenophyta" (104) is debatable.

CLASSIFICATION OF FLAGELLATE AND COCCOID FORMS

Certain differences of opinion as regards classification of the flagellate, palmelloid and coccoid types may well be discussed at this stage. Various authorities (41, 92) do not refer the palmelloid members to distinct orders, regarding them merely as modifications of the flagellate type resulting from the well known tendency of forms like *Chlamydomonas* to assume under certain circumstances a temporary *Palmella*-stage. In both *Chlamydomonas* (Chlorophyceae) and *Chromulina* (Chrysophyceae), species are known in which this palmelloid phase is dominant, although in such instances the individual cells usually retain their flagella so that the motile condition is readily readopted. Fritsch (41, 162), therefore, classes the palmelloid types as Tetrasporineae, Heterocapsineae, Chrysocapsineae, *etc.*, which constitute suborders of the Volvocales, Heterochloridales, Chrysomonadales, *etc.*, the flagellate members being grouped in the suborders Chlamydomonadineae, Heterochlorineae, Chrysomonadineae, *etc.*

A not uncommon modification of the motile flagellate cell, especially in those classes in which it is devoid of an enveloping membrane, is constituted by the permanent adoption of an amoeboid or rhizopodial habit. In his 1914 paper Pascher (95) placed such forms as suborders of Chrysomonadales and Heterochloridales, but in a later communication (104), dealing with the broad classification of algae, they rank as separate orders (*cf.* also 135). Fritsch (41), however, treats them in the same way as the palmelloid types, classing them as Heterorhizidineae, Rhizochrysidineae, Rhizodininineae among Heterochloridales, Chrysomonadales and Dinoflagellata, respectively. Such differences in taxonomic outlook are a matter of individual opinion, but in the view of the writer, inclusion of the palmelloid and rhizopodial forms in the same order as flagellate types affords a better expression of their true affinities and thus a more natural classification.

A number of genera (*e.g., Chlorangium, Prasinocladus = Chlorodendron* Senn) producing colonies with a branched dendroid habit, in which the individual cells readily become detached and assume a temporary motile habit, have long been known among Chlorophyceae. In conformity with the practice adopted by Wille (165), Blackman and Tansley (7) placed them among the palmelloid forms, and this has been followed even in recent times (113, 135). There is, however, no evidence of any direct connection with the palmelloid type, as was first claimed by Oltmanns (90, *136*) who referred these forms to a distinct family, the Chlorodendraceae (Chlorangiaceae of Smith (135)), while Fritsch (41, 162) establishes for them a separate suborder, the Chlorodendrineae, and refers equivalent types among Xanthophyceae (*Mischococcus*) to the Heterodendrineae. Pascher (104, *327*) groups the dendroid green algae in a separate subdivision (Chlorangiales) of his Tetrasporineae. It should be added that among the forms at present classed with *Chlorangium* and *Prasinocladus* among Chlorodendrineae there are several aberrant types (*Ecballocystis, Hormotila*) that may prove to belong to an altogether different affinity, the Hormotilaceae of Pascher (104, *327*).

In those classes in which a wide variety of flagellate genera has become known, the need for a taxonomic grouping has resulted in a diversity of schemes, several of which are open to criticism. Among Volvocales the marked contrast in cell-structure exhibited by certain species of *Sphaerella* (*Haematococcus*) as compared with *Chlamydomonas* (24) has led diverse recent authorities (41, 135, 161) to recognise two separate families, Chlamydomonadaceae and Sphaerellaceae. Better knowledge of *Sphaerella* indicates that it is probably derivative from *Chlamydomonas,* but the two generic types remain sufficiently distinct to warrant reference to separate families. Whether *Chlorogonium* is justifiably referred to Sphaerellaceae remains to be seen (*cf.* 135, *346*).

Stephanosphaera is usually placed in the same family as *Sphaerella,* while there is difference of opinion whether *Volvox* should be referred to it. Many types of *Volvox* certainly differ very profoundly in cell structure from that of the majority of other colonial Volvocales, and the approximation to *Sphaerella* by no means lies only in the presence of rhizopodial processes emanating from the main body of the protoplast. The main series of colonial Volvocales (*Gonium, Pandorina, Eudorina, Pleodorina, etc.*) is referred by many to a family (Volvocaceae) distinct from that harbouring the unicellular forms, but the writer (41) dissents from this view. It may be pointed out that it is hardly logical to include *Stephanosphaera* in Sphaerellaceae and to exclude *Gonium* and other genera from Chlamydomonadaceae. Moreover, there can be no question that most species of *Volvox* differ far more profoundly from the other Volvocaceae than the latter do from *Chlamydomonas.*

The flagellate members of Chrysophyceae exhibit a greater degree of variety in the motile apparatus than do the corresponding types among Chlorophyceae and Xanthophyceae. Senn (128) first introduced the classification of Chrysomonadineae into Chromulineae with one, Hymenomonadeae (Isochrysideae (94)) with two equal, and Ochromonadeae with two unequal flagella, to which in recent times have been added the Prymnesieae (23) with one short and two long flagella. Such a grouping, although taxonomically useful in the present state of our knowledge, is hardly likely to be a natural one. The less specialised members of Isochrysideae and Ochromonadeae are likely to be closely related. The two equal flagella of the former are probably always dissimilar in structure (109, 156), in the same way as are the unequal flagella of the Ochromonadeae and Xanthophyceae (155). It is possible, moreover, that the uniflagellate condition of the Chromulineae may have resulted from loss of a flagellum. Certain species of *Chromulina* certainly approximate closely to certain types of *Ochromonas.* The deficiencies of the existing basis of classification have become particularly patent in relation to the coccoid Chrysophyceae, where vegetatively similar forms commonly propagate by zoospores with contrasting types of flagellation. It seems probable that in the future the flagellate Chrysophyceae will have to be grouped more particularly with reference to their cell structure, especially that of the envelope.

Pascher (95) separated from the main class of the Dinophyceae a series, the Desmokontae, which appear on the whole to show a more primitive organisation and are possibly related to the Cryptomonadineae. They include a number (*Desmomastix, Pleromonas, Haplodinium*) of relatively simple flagellate types (Desmomonadaceae) with a pair of apically inserted, dissimilar flagella, a palmelloid type (*Desmocapsa,* (122, *11*)), as well as the more specialised marine family Prorocentraceae and the Dinophysiales, which last approximate more closely to the Dinoflagellata. Fritsch (41) classes these Desmokontae as a subdivision of the Dinophyceae, the typical members of which are grouped in the Dinokontae. Space does not permit further discussion of the detailed classification of the latter.

Most authorities have assumed a fairly close relationship between the flagellate and coccoid types in the classes of algae at present under consideration. Some (*e.g.,* 8, 113, 161) have in fact included the Volvocales and Chlorococcales in a common group[3], and it can not be denied that a case could be made out for such a practice among both Chlorophyceae and other algal classes. Those coccoid forms that reproduce by zoospores are in part at least closely related to motile flagellate types, and instances of the retention of essential features of the motile individual (stigma, contractile vacuoles) by the coccoid cell are indeed known in practically all classes under discussion. The discovery of such forms among Chlorococcales led Korschikoff (66, *490;* 69) to establish the group Vacuolatae, although this means putting too much stress on a single character; moreover, recent work on *Chlorococcum* (47, *375*) tends to show that the presence of contractile vacuoles may depend on the environment. Despite the obvious affinities, the contrast between the free-moving flagellate and coccoid habits is significant of the evolutionary trend that probably initiated the

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[3] This is called Protococcales. Since it is now almost generally agreed that *Protococcus* (*Pleurococcus*) is a reduced filamentous member allied to the Ulotrichales or Chaetophorales, it is time that the designation Protococcales be altogether abandoned for the coccoid green types and the name Chlorococcales, introduced by Fritsch (162; *cf.* also 135), substituted.

SYNOPSIS OF SOME OF THE MORE RECENT SYST

(The sequence of orders and families is not

West, 1916	Printz, 1927	Oltmanns, 1922
	Euchlorophyceae	Chlorophyceae
Protococcales (Isokontae)	Protococcales	Volvocales
Volvocineae		
Polyblepharidaceae	Volvocaceae	Polyblepharidaceae
Sphaerellaceae		Chlamydomonadaceae
Volvocaceae		Phacotaceae
		Volvocaceae
Tetrasporineae		
Palmellaceae	Tetrasporaceae	Tetrasporaceae
		Chlorodendraceae
Protococcaceae	Chlorococcaceae	Protococcales
Dictyosphaeriaceae	(Protococcaceae)	Protococcaceae
Autosporaceae	Pleurococcaceae	
Chaetopeltidaceae	Protosiphonaceae	Protosiphonaceae
Chlorococcineae	Oocystaceae	Scenedesmaceae
Planosporaceae	Hydrodictyaceae	
Hydrodictyaceae	Coelastraceae	Hydrodictyaceae
	Chlorosphaeraceae	
Ulotrichales (Isokontae)	Chaetophorales	Ulotrichales
Ulotrichaceae	Ulotrichaceae	Ulotrichaceae
Microsporaceae		
Cylindrocapsaceae	Cylindrocapsaceae	Cylindrocapsaceae
	Ulvaceae	Ulvaceae
	Blastosporaceae	Prasiolaceae
Chaetophoraceae	Chaetophoraceae	Chaetophoraceae
Aphanochaetaceae	Aphanochaetaceae	Aphanochaetaceae
Trentepohliaceae	Trentepohliaceae	Trentepohliaceae
Coleochaetaceae	Coleochaetaceae	Coleochaetaceae
	Chaetopeltidaceae	
Ulvales (Isokontae)		
Ulvaceae		
Schizogoniales (Isokontae)		
Prasiolaceae		
Oedogoniales (Stephanokontae)	Oedogoniaceae	Oedogoniaceae

EMS OF CLASSIFICATION OF THE GREEN ALGAE
necessarily that of the respective authors)

Smith, 1933, 1938	Fritsch, 1935	Pascher, 1931[4]
Volvocales	Volvocales	Volvocineae
	Chlamydomonadineae	Chlamydomonadales
Polyblepharidaceae	Polyblepharidaceae	Polyblepharidaceae
Chlamydomonadaceae	Chlamydomonadaceae	Chlamydomonadaceae, etc.
Phacotaceae	Phacotaceae	
Sphaerellaceae	Sphaerellaceae	Volvocales
Volvocaceae		Goniaceae
Spondylomoraceae		Volvocaceae, etc.
Tetrasporales	Tetrasporineae	Tetrasporinae
Coccomyxaceae		Tetrasporales
Palmellaceae	Palmellaceae	Palmellaceae
Tetrasporaceae	Tetrasporaceae	Tetrasporaceae
	Chlorodendrineae	Chlorangiales
Chlorangiaceae	Chlorodendraceae	Chlorangiaceae
		Hormotilaceae
Chlorococcales	Chlorococcales	Protococcineae[5]
Chlorococcaceae	Chlorococcaceae	Protococcaceae
Endosphaeraceae	Eremosphaeraceae	Eremosphaeraceae
Characiaceae	Chlorellaceae	Chlorellaceae
Protosiphonaceae	Selenastraceae	Protosiphonaceae
Scenedesmaceae	Dictyosphaeriaceae	Scenedesmaceae
Oocystaceae	Oocystaceae	Oocystaceae
Hydrodictyaceae	Hydrodictyaceae	Hydrodictyaceae, etc.
Coelastraceae	Coelastraceae	
Ulotrichales	Ulotrichales	Ulotrichineae
	Ulotrichineae	Ulotrichinae
Ulotrichaceae	Ulotrichaceae	Ulotrichales[6]
Microsporaceae	Microsporaceae	Ulotrichaceae
Cylindrocapsaceae	Cylindrocapsaceae	Cylindrocapsaceae
	Ulvaceae	Ulvaceae
	Prasiolineae	
	Prasiolaceae	Blastosporaceae
	Sphaeropleineae	
	Sphaeropleaceae	Chaetophorales
	Chaetophorales	Microthamniaceae
Chaetophoraceae	Chaetophoraceae	Chaetophoraceae
		Aphanochaetaceae
Trentepohliaceae	Trentepohliaceae	Trentepohliaceae
Coleochaetaceae	Coleochaetaceae	Coleochaetaceae
	Chaetosphaeridiaceae	Chaetopeltidaceae
Protococcaceae	Pleurococcaceae	Microsporinae
Ulvales		Microsporales
Ulvaceae		Microsporaceae
Schizomeridaceae		
Schizogoniales		
Cladophorales	Cladophorales	
Cladophoraceae	Cladophoraceae	
Sphaeropleaceae		
Oedogoniales	Oedogoniales	Oedogoniineae
		Oedogoniales
Oedogoniaceae	Oedogoniaceae	Oedogoniaceae

West, 1916	Printz, 1927	Oltmanns, 1922
Siphonales (Isokontae)	Siphonales	Siphonales
Protosiphonaceae		
Bryopsidaceae	Bryopsidaceae	Bryopsidaceae
Caulerpaceae	Caulerpaceae	Caulerpaceae
Derbesiaceae	Derbesiaceae	Derbesiaceae
Codiaceae	Codiaceae	Codiaceae
Phyllosiphonaceae	Phyllosiphonaceae	
Vaucheriaceae	Vaucheriaceae	Vaucheriaceae
Chaetosiphonaceae		
Siphonocladales (Isokontae)	Siphonocladales	Siphonocladales
Cladophoraceae	Cladophoraceae	Cladophoraceae
Sphaeropleaceae	Sphaeropleaceae	Sphaeropleaceae
		Siphonocladaceae
Valoniaceae	Valoniaceae	Valoniaceae
Dasycladaceae	Dasycladaceae	Dasycladaceae
Conjugatae (Akontae)	Conjugatae	Conjugatae
Desmidiaceae	Desmidiaceae	Mesotaeniaceae
Saccodermae	Saccodermae	
Placodermae	Placodermae	Zygnemataceae
Zygnemaceae	Zygnemataceae	
		Desmidiaceae
	Charophyta	Charales

sedentary habit which is dominant among plants, and for that reason the coccoid forms are best referred to separate orders.

The great diversity exhibited among the coccoid green algae has led to the establishment of a large number of genera whose exact interrelationships are difficult to trace, especially as our knowledge of the life-history of many of them is still meagre. As a consequence no two authorities are agreed as to their classification into families, but little purpose would be served by discussing the different schemes here. In all series of coccoid forms (96), and very noticeably among Chlorophyceae, a more or less appreciable number seem to have completely abandoned the flagellate condition so that the daughter individuals never pass through a motile phase and acquire their mature characteristics before liberation from the membrane of the parent (so-called autospore formation). Brunnthaler (18) first utilised this feature in the classification of the Chlorococcales by subdividing the order into Zoosporinae and Autosporinae, according as reproduction was effected with the aid of zoospores (or motile gametes) or not. Oltmanns (92) and others

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[4] Supplemented in parts from the "Süsswasserflora".

[5] After Brunnthaler (19).

[6] After Heering (55).

Smith, 1933, 1938	Fritsch, 1935	Pascher, 1931[4]
Siphonales	Siphonales	Siphonineae
		Monosiphonae
Halicystaceae	Protosiphonaceae	Valoniales
Bryopsidaceae		
Caulerpaceae	Caulerpaceae	Caulerpales
Derbesiaceae	Derbesiaceae	
Codiaceae	Codiaceae	
Phyllosiphonaceae	Phyllosiphonaceae	Vaucheriales
Vaucheriaceae	Vaucheriaceae	Plectenchymatae
	Chaetosiphonaceae	Codiales
Siphonocladales		Siphonocladineae
		Radiatae
		Siphonocladales
		Dasycladales
		Cladophorinae
Valoniaceae	Valoniaceae	Cladophorales
Dasycladaceae	Dasycladaceae	Sphaeropleinae
Zygnematales	Conjugales	Conjugatae
	Euconjugatae	Saccodermae
	Mesotaenioideae	Mesotaeniales
Mesotaeniaceae	Mesotaeniaceae	
	Zygnemoideae	Zygnemales
Zygnemataceae	Zygnemataceae	
	Mougeotiaceae	
	Gonatozygaceae	
	Desmidioideae	Placodermae
Desmidiaceae	Desmidiaceae	
Charophyceae	Charales	Charophyta

have accepted this as the basis of classification among Chlorococcales, but, although it is convenient from a taxonomic standpoint, it may be questioned whether it affords a natural grouping. The step from the zoosporic to the autosporic condition may be induced by a change of environment; thus, the zoosporic *Chlorococcum* in cultures often multiplies without intervention of zoospores, while those forms of the zoosporic *Trebouxia* that constitute the algal partners of lichens likewise propagate by formation of autospores (aplanospores). The autosporic condition may well have originated in various evolutionary series among the coccoid green algae, and their segregation into Zoosporinae and Autosporinae probably obscures the actual affinities (*cf.* also 135, *465*). In particular there is reason to suspect that the coenobial members of these two series (Hydrodictyaceae, Coelastraceae comprising forms like *Coelastrum, Scenedesmus, etc.*) are more closely interrelated than their reference to two suborders would imply. Moreover, in other classes (Xanthophyceae, Dinophyceae) a comparable separation of zoosporic and autosporic forms has proved to be altogether impracticable. It is probable, therefore, that with increasing knowledge of details of reproduction a different grouping of Chlorococcales will be adopted.

CLASSIFICATION OF THE REMAINING CHLOROPHYCEAE

At the time when the importance of the Flagellata as a phase in algal evolution was first recognised, several attempts were made to separate other series from the large remaining mass of the Chlorophyceae. Bohlin (14, *25*) grouped the Oedogoniales as Stephanokontae, implying an origin for these forms from a flagellate stock with a ring of flagella, although he retained the Stephanokontae as a subdivision of Chlorophyceae. Blackman and Tansley (8, *44*), however, grouped them as a separate class (*cf.* also 161), and the same procedure was adopted with respect to the Conjugatae (Conjugales) which were named Akontae (8, *45*). Diverse of the earlier authorities (90, 165) separated the Conjugatae from the remaining Chlorophyceae. Adoption of the classes Stephanokontae and Akontae was challenged by Fritsch (37, 38) on the grounds that their separation from other Chlorophyceae "must obscure the essential principles underlying the present-day concept of algal evolution, since in the pigmentation of their chloroplasts, in the possession of pyrenoids with a 'starch-sheath', in the storage of starch, and the chemical nature of their cell-walls, these two groups are altogether like other Chlorophyceae". In recent systems of classification (41; 100, *19;* 135; 162) the classes Stephanokontae and Akontae have been abandoned and the two sets are grouped in the orders Oedogoniales and Conjugatae or Conjugales (Zygnematales of Smith) among Chlorophyceae. Oltmanns (92) and Printz (113), however, still subdivide the green algae into two main divisions, the Euchlorophyceae and Conjugatae of the latter. The Conjugatae show many peculiarities, but they can hardly be regarded as exhibiting a greater measure of distinguishing characteristics than do the Oedogoniales or Siphonales, and it may be doubted that the attitude of Oltmanns and Printz is justified. In Oltmanns' treatment the diatoms are interposed between consideration of the Conjugatae and that of the remaining Chlorophyceae, but, despite a certain degree of parallelism, this juxtaposition of Conjugales and Bacillariales is quite unwarranted, since there can be no affinity between these two groups.

The Chlorophyceae comprise a larger number of filamentous and simple thalloid forms than are to be met with in any other class of algae, and marked differences of opinion have arisen as to their grouping. The writer (41, *73*) recognises, apart from Oedogoniales and Conjugales, only three orders of filamentous Chlorophyceae—Ulotrichales, Cladophorales, Chaetophorales. All other authorities include the Chaetophorales in the Ulotrichales, called Chaetophorales by Wille (166) and Printz (113), although the numerous genera comprised in Fritsch's Chaetophorales are invariably placed in families (Chaetophoraceae, Trentepohliaceae, *etc.*) distinct from those harbouring the true Ulotrichales. Reference of the former to a separate order by the writer turns on the recognition of the great phylogenetic importance of their special habit which has been designated as heterotrichous (38, *111*). In typical representatives of Chaetophorales (*Stigeoclonium, Trentepohlia, Coleochaete pulvinata*) the plant body is developed as two distinct systems, a primarily formed prostrate one and a secondarily formed erect one. An altogether comparable differentiation of the plant body is met with in the simpler filamentous brown and red algae and is also recognisable among blue-green algae (42, 44). The Chaetophorales are in fact in vegetative habit parallel to the simpler members of Ectocarpales and Nemalionales among Phaeophyceae and Florideae, respectively, while forms parallel to the Ulotrichales are not found in these orders. Among Phaeophyceae and Rhodophyceae many of the more specialised forms pass through a primary heterotrichous stage, and it is evident that this habit has afforded an important starting point for the development of larger and more highly differentiated types of algal thalli.

While the families Chaetophoraceae, Trentepohliaceae and Coleochaetaceae are in general well defined, a group of aquatic genera (*Gongrosira, Ctenocladus, etc.*), commonly classed as Gongrosireae (Leptosireae of Printz (113)), as well as the endophytic and usually lime-boring Gomontieae, are difficult to place. Smith (135) and Fritsch (41, *280*) class them among Trentepohliaceae, an otherwise essentially terrestrial group; this was the practice at first adopted (165) but later (166) abandoned by Wille. Both he and Printz (113) include the Gongrosireae and Gomontieae in the Chaetophoraceae (*cf.*also 161). As compared with the typical members of the latter, the Gongrosireae and Gomontieae are distinguished by an absence of hairs and more especially by a usual differentiation of distinct sporangia. They afford evidence of a greater degree of specialisation and in various respects certainly approach more closely to the terrestrial Trentepohlieae. It might be better until such time as they have been more fully studied to place them in a separate family.

As in other heterotrichous series, the Chaetophorales comprise a considerable number of reduced and in part unicellular forms (*Chaetosphaeridium, Dicranochaete, etc.*) whose exact affinities are, in the present state of our knowledge, difficult to assess. They are usually grouped in a single family, the Chaetosphaeridiaceae of Fritsch (41), although this constitutes an altogether artificial assemblage of forms. Smith's (135, *411*) reference of them to Coleochaetaceae can scarcely be justified, while West's (161) inclusion of them among Protococcales obscures their undoubted affinities with Chaetophorales. West (*cf.* also 113) classes them with *Chaetopeltis* as Chaetopeltidaceae, but, although the genus *Chaetopeltis* possibly includes types that should be referred to Chlorococcales (70), the *Chaetopeltis* of Berthold (5) is no doubt a member of Chaetophoraceae (41, *260;* 135, *402*; 22). *Pleurococcus* (*Protococcus*) is now generally recognised as an extremely reduced genus, possibly related to some of the Trentepohlieae.

Wille (165), Oltmanns (92) and Printz (113) group all filamentous Chlorophyceae, including the Oedogoniaceae but excluding the Zygnemales and Cladophoraceae, in one comprehensive order, variously styled Chaetophorales or Ulotrichales (*cf.* also 104). Setchell and Gardner (131) and Smith (135), on the other hand, segregate from the Ulotrichales, apart from the Oedogoniales, the two orders Ulvales and Schizogoniales, the former first distinguished by Blackman and Tansley (8), the latter first established by G. S. West (159; *cf.* also 20). Reference of the forms comprised in these two series to separate orders is a matter of opinion. The Ulvaceae undoubtedly show a vegetative advance over other Ulotrichales, and they differ also in the possession of an isomorphic life-cycle. On the other hand, the early stages of *Ulva* and *Enteromorpha* are filamentous, and such stages, especially in the latter, closely resemble *Ulothrix;* moreover, there is great resemblance in details of reproduction. Smith (135, *457; cf.* also 67) refers to the Ulvales, also a family Schizomeridaceae, in which he includes the genus *Schizomeris,* an alga of doubtful status which many have regarded but as a developmental state of *Ulothrix* (*cf.* 41, *201*). Fritsch (41) classes the Ulvaceae in the suborder Ulotrichineae side by side with the Ulotrichaceae (*cf.* also 149) to which he refers *Schizomeris.*

A better case can be made out for referring the Prasiolaceae (Blastosporaceae of Printz (113) and Heering (55)) to the separate order Schizogoniales (Prasiolales (65, 149)). In the possession of an axile chloroplast and the apparent absence of motile stages during reproduction, which is largely vegetative, they stand markedly apart from other Ulotrichales. Forms with an axile chloroplast are known among coccoid (*Trebouxia*) and palmelloid (*Asterococcus*) green algae, although in them multiplication is effected by motile elements; moreover, certain species of *Chlamydomonas* have axile chloroplasts (103), although these are rather different in type from those of the other green algae mentioned. It may be that better knowledge of Prasiolaceae will lead to recognition of a definite evolutionary series among Chlorophyceae comprising forms with axile chloroplasts, and there is a remote possibility that the Conjugales in which the chloroplasts are predominantly axile and reproduction by motile elements is lacking, might be connected with this line. Fritsch (41) includes the Prasiolaceae in the suborder Prasiolineae among Ulotrichales.

Bohlin (14, *25*) referred the genus *Microspora,* distinguished by its walls being composed of H-shaped pieces as in *Tribonema* and diverse other Xanthophyceae, as well as by the absence of pyrenoids, to a distinct order, but most recent authorities accept a near relation to the Ulotrichaceae. Pascher (104, *327*), however, places *Microspora* in a separate division (Microsporineae) of his Ulotrichineae (*cf.* also 55, *146*) and suggests a possible relationship to certain Chlorococcales with a cell wall composed of two pieces.

Many systematists recognise two orders of siphoneous green algae, the Siphonales and the Siphonocladales (92, 100, 113, 131, 135, 161, 166), although Blackman and Tansley (8) grouped them as two suborders (Siphoneae, Siphonocladeae) in the single order Siphonales. The Siphonocladales[7] comprise numerous genera in

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[7] Most of those who recognise the order Siphonocladales include the Dasycladaceae in it. The origin of this peculiar error is obscure. In *Dasycladus, Cymopolia, Acetabularia, etc.* the body is one continuous coenocyte and no septation of any kind is recognisable in the vegetative region.

which the mature thallus consists of few or many multinucleate segments, and to them most authorities refer the Sphaeropleaceae and the Cladophoraceae. Fritsch (162, *151*) was the first to depart from this practice, the two families last mentioned being referred to separate suborders of the Ulotrichales (*cf.* also 135), while subsequently (41) the Cladophoraceae were placed in a distinct order, the Cladophorales. Such an order is likewise recognised by Smith (136), although he includes in it also the Sphaeropleaceae. Pascher (104, *328,* footnote) points out that the Cladophoraceae and Sphaeropleaceae are probably not related to the main series of the Siphonocladales and that they are likely to have quite a different affinity.

The reference by Fritsch of the genus *Sphaeroplea* to the suborder Sphaeropleineae of Ulotrichales is based on a survey of all its morphological features (39). Relevant are (*a*) the annular chloroplasts, closely resembling those of *Ulothrix zonata,* although more complex reticulate types develop in certain species in later stages, (*b*) formation of the sexual cells (ova and spermatozoids) in the ordinary segments of the filaments, (*c*) liberation and reception of the male cells through small apertures in the wall, one commonly situated near each point of occurrence of a primary annular chloroplast unit, and (*d*) the haploid life-cycle. *Sphaeroplea* is regarded as an advanced *Ulothrix*-like type in which septation is retrogressive. There is some indication, moreover, that there may exist sphaeropleaceous types which have not attained the oogamy exhibited by the ordinary species (*Sphaeroplea tenuis* (39, *18*)). It is difficult to detect any affinities to Cladophoraceae.

The members of this family are unbranched (*Urospora, Chaetomorpha*) or richly branched (*Cladophora*) filamentous types, many of which appear to have advanced to an isomorphic life-cycle. The usually large cells contain numerous nuclei and an often complex reticulate chloroplast which, in certain species, apparently later fragments into many separate pieces, often still retaining the primary reticulate arrangement. The young plants are filamentous from the first and the germlings formed by zoospores and zygotes undergo progressive septation by cross walls which arise as annular ingrowths from the longitudinal walls (17). Zoospores and gametes are formed in large numbers in the ordinary vegetative cells and are liberated through a lateral aperture, as in *Ulothrix.* Germlings of *Urospora* have a curved plate-like parietal chloroplast, closely resembling that of Ulotrichaceae and Ulvaceae, and the young cells harbour but few nuclei (114, *280*). The cells of some species of *Rhizoclonium* permanently possess only a few nuclei (110). For these diverse reasons the Cladophorales are regarded as filamentous types in which elaboration of the cells and appearance of a coenocytic character is secondary. They may have originated from a ulotrichaceous stock.

After removal of the Sphaeropleaceae and Cladophoraceae there remain among Siphonocladales a large number of marine genera which are commonly referred to a number of separate families (11, *18*); certain of them (*Cladophoropsis, Boodlea*) exhibit considerable superficial resemblance to *Cladophora*. There are, however, marked points of difference. The germlings are vesicular, and more or less closely resemble *Valonia* which is usually regarded as the most unspecialised member of this series. The division into multinucleate segments is accomplished, moreover, in an entirely different manner by a process styled "segregative division" by Boergesen (10, *34*). The protoplasm of the coenocyte contracts and undergoes simultaneous division into a number of multinucleate masses, each of which becomes surrounded by a membrane independent of that of the parent coenocyte. Subsequently the segments enlarge and become pressed against one another so that the double membranes delimiting them may be difficult to recognise; the segments may again divide in the same manner. In various genera (*Siphonocladus* (9), *Cladophoropsis, etc.*) the segments tend to assume a linear arrangement, and some of them grow out as branches, giving the cladophoraceous habit referred to above. Segregative division has been established for *Valonia, Dictyosphaeria, Siphonocladus, Cladophoropsis* and *Struvea,* besides others, but it has not been shown to occur in *Microdictyon* and *Anadyomene*. It has recently been found (58, 59) that these two genera possess an isomorphic life-cycle, the diploid phase producing quadriflagellate zoospores and the haploid biflagellate isogametes. The type of life-cycle, contrasting so markedly with the diploid one now established for a considerable number of Siphonales (including *Valonia* (126)), raises the question whether these two genera are correctly associated with the other segmented Siphonales (43, *537*), and it is possible that they should be referred to Cladophorales.

However that may be, it is difficult to find any clear indications of affinity between the other Siphonocladales and the Cladophorales. Fritsch (41, *424*) has suggested that segregative division may be interpreted as a process of cyst-formation in which the cysts are retained and germinate *in situ*. On this view the series of genera exhibiting this mode of segmentation can be regarded as variously developed colonial types, in which the unit is a coenocyte. The peculiar process of segregative division represents one of the many diverse ways in which the siphoneous green algae have evolved into larger thalli with considerable mechanical stability. The writer can recognise no reasons for placing the genera under discussion in a separate order and is of the opinion that they should be included in one or more families among Siphonales.

Feldmann (33) has opposed removal of the Cladophoraceae from the segmented Siphonales, but this is not the place to enter into a detailed discussion of his position, with which the writer will deal elsewhere. The chloroplast characters upon which he lays particular stress can be regarded only as of subsidiary importance.

The Vaucheriaceae, the only known oogamous Siphonales, have been a source of much perplexity to systematists. *Vaucheria* has been suspected of being a member of Xanthophyceae, and the Vaucheriaceae were indeed placed in that class by Blackman and Tansley (8, *58*). This was based mainly on the occurrence of oil as the photosynthetic product, which has been stated (12, *30;* 113, *328*) to go hand in hand with the presence of an excess of xanthophyll in the chloroplasts, and on the mode of arrangement of the flagella on the spermatozoids, which is unlike that characteristic of Chlorophyceae. Pascher (56, *69,* footnote) also suspects inequality of the pairs of flagella on the compound zoospore. Except for the presence of oil, all these characters are of dubious value for assigning to *Vaucheria* a position among Xanthophyceae. The presence of excess xanthophyll has never been adequately proved and the arrangement of the flagella on the spermatozoids is as little typical of the Xanthophyceae as it is of Chlorophyceae. Discovery of the starch-producing *Dichotomosiphon* (31), with sex organs comparable in type to those of *Vaucheria,* went far to establish a chlorophycean affinity for the two genera and, in view of the vegetative resemblances between *Dichotomosiphon* and the filaments of Codiaceae, to support an inclusion among Siphonales. The genera

Vaucheriopsis and *Pseudodichotomosiphon* (41, *428*)), established during the last 20 years, have not helped in the elucidation of affinities. Dangeard's (26, *184*) discovery of the presence of pyrenoids in certain marine species of *Vaucheria* is also of no assistance in this connection, since such structures occur also in some Xanthophyceae.

The possibility that the resemblances between *Vaucheria* and *Dichotomosiphon* may be homoplastic must be envisaged, and the true affinities of the former can not be held conclusively proved until a more detailed study of zoospores and spermatozoids has been undertaken and the proportions of the chloroplast pigments adequately established. It is now almost a platitude to point out that nearly all oogamous green algae occupy very isolated positions, which renders elucidation of their affinities a matter of considerable difficulty.

The status of the Conjugales (Zygnematales of Smith) has already been considered. Detailed classification of the filamentous members presents considerable difficulties, but can not profitably be discussed here. Desmids, comprising a wide diversity of unicellular and colonial types, have long been classed in the two series Saccodermae and Placodermae, the latter including the great bulk of the species. The Saccodermae possess a simple cell wall of a single piece and in general exhibit less specialised types of chloroplasts than the Placodermae, where the wall consists of two overlapping pieces and is perforated by numerous pores. While earlier authorities (87; *cf.* also 113, 161) accepted the two series of desmids as closely allied, Oltmanns (90, *53*) first emphasised the probable primitive character of the Saccodermae (his Mesotaeniaceae), which he regarded as most closely related to the ancestral type from which the Placodermae and filamentous Conjugales were supposed to have diverged as separate lines. In adopting this position he laid stress on the fact that several genera of Mesotaeniaceae (*Mesotaenium, Cylindrocystis*) produce four individuals from their zygotes, while in Placodermae there are usually two and in filamentous Conjugales only one, the supernumerary nuclei aborting. Later researches have shown that these numbers are not invariable and that there are also Placodermae producing four individuals from the zygote (153), while two are occasionally formed in *Mesotaenium* (146, 160) and *Spirotaenia* (61, *765*), and this appears to be the rule in *Netrium* (111, *669*). There is evidence of a fairly close affinity between the Saccodermae and the filamentous forms, but the relation of the highly specialised Placodermae to the others is not clear; in particular, their complex wall structure separates them sharply from the other two series. The writer (41) has therefore grouped the Conjugales in two suborders, Euconjugatae and Desmidioideae, distinguishing among the former the two series Mesotaenioideae and Zygnemoideae. Pascher (104) has adopted a similar grouping (*cf.* also 25, *32*).

Oltmanns (90, *52*) was also the first to separate the genera *Gonatozygon* and *Genicularia* from the desmids and to interpret them as filamentous members with a marked tendency to fragment into individual cells. As in other filamentous Conjugales, only a single individual is formed from the zygote. Fritsch (41) follows Oltmanns and refers these genera to the family Gonatozygaceae of Zygnemoideae. Smith (135), on the other hand, retains *Gonatozygon* and *Genicularia* among Mesotaeniaceae. So far no basis has been found for classing the Placodermae in more than one family, and indeed the limits of many genera are so ill defined (40) that it is unlikely that any such grouping could be achieved.

The Characeae, which have a more highly differentiated vegetative system than is found in the multicellular Chlorophyceae and possess highly complex sex organs, are regarded by most as belonging to a separate class of algae (Charophyceae (135)) or even to a separate subdivision of the vegetable kingdom (Charophyta). Fritsch (41), on the other hand, groups them as an order of Chlorophyceae (Charales). The attitude taken depends on the particular characteristics that are stressed, but it is well to realise that there is no evidence that the Characeae differ from the Chlorophyceae either in the nature and proportions of their photosynthetic pigments or in their metabolism; the spermatozoids, though highly specialised, are, moreover, isokontan in type and the life-cycle is haploid.

CLASSIFICATION OF THE PHAEOPHYCEAE

During the early part of the present century the classification of this class initiated by Kjellman (63) in the "Natürliche Pflanzenfamilien" dominated the outlook. Oltmanns (90, *348;* 91, *19*), following him, grouped the brown algae into (*a*) Phaeosporeae (150, *216*), propagating by zoospores and sometimes by motile gametes;

(*b*) Acinetosporeae, distinguished by usual propagation by large motionless monospores; and (*c*) Cyclosporeae, characterised by oogamous reproduction. The first of these subdivisions included the numerous diverse forms comprised in the so-called Ectocarpaceae, as well as the Cutleriaceae, the Sphacelariaceae and Laminariaceae, the second the Tilopteridaceae, and the third the Fucaceae and Dictyotaceae, although Kjellman (63), unlike later authors, excluded the Dictyotaceae from the Phaeophyceae. A similar arrangement was adopted by Setchell and Gardner (132), although they substituted the name Aplanosporeae for the Acinetosporeae of Oltmanns and included in this group, characterised by possession of motionless asexual reproductive cells, both Tilopteridaceae and Dictyotaceae.

The rapid strides in the elucidation of the life-cycles of Laminariaceae and subsequently of diverse genera of Ectocarpales during the second and third decades of this century showed that oogamous sexual reproduction was more widespread than had hitherto been supposed and rendered Kjellman's grouping untenable. A new classification of the class into a number of orders of equal rank was proposed by Kylin (74, *308*) and adopted with considerable amplifications by Oltmanns (93) in the second edition of his book. The latter recognised seven orders: Ectocarpales, Sphacelariales, Cutleriales, Laminariales, Tilopteridales, Dictyotales and Fucales. With the exception of the first, these main subdivisions have been retained by later authorities.

Sauvageau's demonstration (119) that *Dictyosiphon* possesses a heteromorphic life-cycle, the zoospores produced in its unilocular sporangia producing minute filamentous gametophytes propagating by isogametes, afforded a new character for the subdivision of the large and unwieldy group of the Ectocarples, from which the Cutleriaceae had already been removed by Oltmanns (93). Taylor (147) proposed reference of the Dictyosiphonaceae to a separate order, Dictyosiphonales (*cf.* also 144), while a few years later Sauvageau (120) referred the Sporochnaceae, in which he had established a heteromorphic alternation analogous to that of Dictyosiphonaceae, though seemingly with oogamous reproduction of the gametophyte, to a distinct order, the Sporochnales.

More recently still, Kylin (80, *91; cf.* also 148) has grouped the bulk of the Phaeophyceae into Isogeneratae with isomorphic, and

Heterogeneratae with heteromorphic alternation, and distinguishes in each of these main subdivisions a number of orders. Among Isogeneratae he includes the Ectocarpales (*sens. limit.*), Sphacelariales, Cutleriales, Tilopteridales and Dictyotales, among Heterogeneratae the Laminariales, Sporochnales, Desmarestiales, Chordariales, Dictyosiphonales and Punctariales; the last three, all with isogamous sexual reproduction and included in Oltmanns' Ectocarpales, are distinguished only by vegetative characteristics. Sporochnales and Desmarestiales, likewise classed in Ectocarpales by Oltmanns, have gametophytes with oogamous sexual reproduction. The Fucales, in which a true phase-alternation is lacking, constitute a third subdivision of Phaeophyceae (Cyclosporeae) in Kylin's scheme. This classification is adopted by Taylor (149) and G. M. Smith (136).

Kylin's order Ectocarples is restricted to those members of Oltmanns' order that show isomorphic alternation, namely, the filamentous Ectocarpaceae and some of the Myrionemataceae (*Nemoderma, Lithoderma*). Certain genera at present usually included in the latter family appear to be heteromorphic, and are regarded by Kylin as probably reduced Mesogloeaceae (his Chordariaceae, order Chordariales), which may well be so. The writer (43, *545*) has given considerable evidence for the view that the heteromorphic life cycle prevalent among the majority of the more specialised genera belonging to the Ectocarpales of Oltmanns is derived, by divergent development of the two generations, from an isomorphic alternation, comparable to that exhibited by the Ectocarpaceae. The same interpretation is advanced for the origin of the heteromorphic life-cycle of *Cutleria* (42, *409*), and since Kylin refers the Cutleriales to his Isogeneratae, he evidently holds the same view. If, however, such a markedly heteromorphic type as *Cutleria* is associated with the isomorphic *Zanardinia,* with which it is no doubt closely allied, in the same order of Isogeneratae, then the heteromorphic Chordariales, Dictyosiphonales and Punctariales should also be included with Kylin's Ectocarpales in a common order, since their heteromorphic life cycle is just as clearly derived from an isomorphic one as is that of *Cutleria.* Moreover, there is considerable evidence (1; 2; 132, *550*) that the Pacific genus *Heterochordaria,* which in vegetative structure approximates sufficiently to *Chordaria* to have been formerly included in that genus, possesses an isomorphic life cycle. In other words, it seems that this type of life cycle is not altogether confined to the filamentous Ectocarpaceae and the crust-forming Myrionemataceae, but exists also among some of the more highly elaborated types which on the basis of their vegetative structure would be included in Kylin's Chordariales.

The genera of Chordariales, Dictyosiphonales and Punctariales share with the Ectocarpales an essentially isogamous sexual reproduction and the heterotichous habit that is invariably displayed during early stages of development in the sporophyte (42, 402); as regards the Punctariales, too, there is a close linkage with the filamentous Ectocarpaceae by way of such genera as *Phloeospora* and *Isthmoploea*. For these various reasons the writer in the second volume of his book "The Structure and Reproduction of the Algae" has adopted the more comprehensive conception of the Ectocarpales taken by Oltmanns, although segregating from them the oogamous Sporochnaceae and Desmarestiaceae (*cf.* also 45, 53). He recognises nine orders of Phaeophyceae: Ectocarpales, Tilopteridales, Cutleriales, Sporochnales, Desmarestiales, Laminariales, Sphacelariales, Dictyotales and Fucales.

Kylin's Chordariales comprise a large proportion of the haplostichous Ectocarpales of Kuckuck (71), while Dictyosiphonales and Punctariales are the polystichous Ectocarpales of the same authority. The limits of the Chordariales are indicated in the valuable survey recently published by Kylin (82), in which a progressive restriction of meristematic activity, culminating in the Spermatochnaceae with apical growth, is described. The only essential difference between Dictyosiphonales and Punctariales lies in the more or less clearly marked apical growth of the former, and it is not improbable that they may possess the same relation to the Punctariales as the Spermatochnaceae do to other Chordariales. The objection to the recognition of any of these as separate orders is that it obscures their close relation to other Ectocarpales; moreover, the differences between them and the latter are not of the nature usually accepted for distinguishing major systematic units.

It would be going too far to consider the detailed grouping of the genera of Ectocarpales (*sens. lat.*). There is rather general agreement about the limits of many families recognised at present[8],

[8] Such are: Ectocarpaceae, Myrionemataceae, Elachistaceae, Mesogloeaceae (Chordariaceae), Acrotrichaceae, Spermatochnaceae, Punctariaceae, Asperococcaceae, apart from the polystichous families referred to above.

and the chief differences of opinion are encountered in relation to the grouping of the polystichous (parenchymatous) forms where relatively few serviceable systematic characters are now available. All authorities recognise the family Dictyosiphonaceae, and most have accepted the family Encoeliaceae established by Oltmanns (93) for a diversity of parenchymatous genera (*Soranthera, Colpomenia, Chnoospora*) whose only other common characteristic is the production of sporangia around groups of hairs which are often sunk in a depression of the thallus surface. Although this family is almost certainly artificial (*cf.* especially 121, *326*), no more satisfactory basis of classification has so far been found (*cf.* however, 132, *522*).

Exclusion of Cutleriales, Sporochnales and Desmarestiales from the Ectocarpales is warranted not only in possession by the members of each order of a distinctive vegetative organisation and of marked specialisation in reproduction, but also by elimination of the early heterotrichous stages that are so characteristic of Ectocarpales. The marked oogamy of *Desmarestia* and the far-going resemblances between its gametophytes and those of Laminariales, led Schreiber (125), who first elucidated these matters, to propose an inclusion of Desmarestiaceae in Laminariales, but this has rightly not been followed since in vegetative characteristics there are very profound contrasts between the two orders. The uniformity in general organisation among the members of Sphacelariales has long led to their recognition as a coherent unit among Phaeophyceae. Classification of the order into Sphacelariaceae, Stypocaulaceae and Cladostephaceae by Oltmanns (93), on the basis of Sauvageau's comprehensive studies (118), has been generally accepted. Nor are there any differences of opinion about the limits of the Dictyotales.

Discovery of the gametophytes of *Chorda* resulted in transference of this genus from the Ectocarpales, among which it was formerly classed, to the Laminariales, where it occupies a family of its own (Chordaceae) on the grounds of its simple vegetative structure. The remaining Laminariales are now usually grouped in the three families Laminariaceae, Lessoniaceae and Alariaceae, established by Setchell (129). The diverse taxonomic series distinguished by Oltmanns among Fucales (89, 93) have been given the rank of families by the writer in his forthcoming book (46); he distinguishes Fucaceae (*Fucus, Pelvetia, Ascophyllum, Seirococcus, etc.*), Himanthaliaceae (*Himanthalia*), Cystoseiraceae (*Pycnophycus, Halidrys, Cystoseira, etc.*), Sargassaceae (*Sargassum, Coccophora, etc.*), Hormosiraceae (*Notheia, Hormosira*), Durvilleaceae (*Durvillea*) and Ascoseiraceae (*Ascoseira*). The last of these (134, *50*) awaits further investigation before its exact affinities can be determined, while the relation of the Durvilleaceae to the other families remains obscure. It may be mentioned here that the genus *Splachnidium*, often doubtfully included in the Fucales in the past, has now been shown (71, 133) to be a specialised member of the haplostichous Ectocarpales.

CLASSIFICATION OF THE RHODOPHYCEAE

A broad division of this class into Bangioideae (Bangiales) and Florideae has long been recognised (124). The principal distinctions are the lack of differentiated carpogonia and the direct division of the zygote into carposporangia in the former; features of lesser importance are the absence of pit-connections between the cells, diffuse growth and frequent presence of axile chromatophores in the Bangioideae. The latter are a small group comprising a number of marine filamentous (*Erythrotrichia*) and parenchymatous genera (*Bangia, Porphya*), as well as a rather varied assortment of largely freshwater or terrestrial palmelloid types (*Asterocytis, Porphyridium, Chroothece*) which are usually regarded as reduced forms. Rosenvinge (116, *56*) grouped the genera as Bangieae, Erythrotrichieae and Goniotrichieae (*cf.* also 142), whilst the writer (46) recognises two families, Bangiaceae (with Bangieae and Erythrotrichieae) and Porphyridiaceae (with *Goniotrichum, Asterocytis, Porphyridium, etc.*) (*cf.* also 81, *39*). In both families genera with one (*Kyliniella, Porphyropsis*) or more (*Rhodospora*) parietal chromatophores are known. It is possible that, as is true of some desmids (40), these are derivative forms in which the central part of the axile chromatophore typical for the members of this subclass has been suppressed, while its peripheral processes have become enlarged.

The relatively simple vegetative organisation, the simple sex organs, and the direct germination of the zygote led Rosenvinge (117) to regard the Bangioideae as primitive among Rhodophyceae and to suggest the name Protoflorideae for them, a name which has been adopted in a few quarters. It must be realised, however, that the direction of vegetative development among the Bangiaceae is quite different from that among the Florideae. The former exhibit a marked parenchymatous tendency and possess diffuse growth, while the Florideae are pseudoparenchymatous uni- and multiaxial forms with apical growth. True, in both series the least specialised genera (*Erythrotrichia, Acrochaetium*) in part possess a heterotrichous filamentous habit (42, *399*), but that is clearly a step in evolution that was reached in all classes of algae that passed beyond the simple filamentous stage. The exact degree of relationship between Bangioideae and Florideae is not at present clear, but, whatever it may amount to, it is unlikely that the two subclasses represent more than a divergent development in quite different directions from a common ancestry (*cf.* also 81, *55*).

Among the many diverse genera included in Florideae, a distinction between those forming gonimoblast threads (which produce the carposporangia) directly from the carpogonia and those forming these threads from other adjacent or distant cells (auxiliary cells) after fertilisation was already recognised by Bornet and Thuret (15, *159*) and more clearly stated by Schmitz (123). The genera showing the former feature (*Acrochaetium* = *Chantransia, Batrachospermum, Nemalion,* etc.) are classed in the order Nemalionales (124), the limits of which have become more sharply defined as a result of the demonstration by Svedelius in 1915 (143) that in *Scinaia* the first divisions in the germinating zygote are meiotic so that the gonimoblasts and carpospores contain the haploid number of chromosomes. This haplobiontic condition was subsequently established also in *Nemalion, Batrachospermum* and other members included in Nemalionales, while *Atractophora* and *Naccaria* (*76, 11*), *Bonnemaisonia* (*72*; *76, 21*) and *Asparagopsis* (145), formerly classed in other orders of Florideae, were shown by Kylin and Svedelius to belong to Nemalionales.

This order in its present limits exhibits an appreciable range in vegetative construction from simple filamentous genera like *Acrochaetium* to relatively specialised uni- (*Bonnemaisonia, Asparagopsis*) and multiaxial (*Scinaia*) forms. Hand in hand with this, but not exactly parallel to it, goes an increasing complexity in the post-fertilisation events. Kylin (*76, 114;* 81, *57*) consequently recognises a considerable number of families[9] differing in vegetative and reproductive features. It is significant that in the more specialised uni- (Bonnemaisoniaceae) and multiaxial (Chaetangiaceae) families there is a tendency for the diploid nucleus, prior to meiosis, to pass into a cell underlying the carpogonium, for differentiation of special nutritive cells or threads in the neighbourhood of the latter, and for development of a sterile envelope around the gonimoblast. These are features met with in the other series, the diplobiontic Florideae considered below, and the facts imply that a pronounced evolutionary advance, parallel to some extent with that found among diplobiontic forms, has occurred also among the haplobiontic Nemalionales, and that the two large series of Florideae represent divergent developments from some common source. So far as present evidence goes, all Nemalionales pass through a typical heterotrichous stage during early development (43, *400*).

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[9] Acrochaetiaceae, Batrachospermaceae, Lemaneaceae, Naccariaceae, Bonnemaisoniaceae, Helminthocladiaceae, Chaetangiaceae; of these, the last two comprise the multiaxial genera.

Schmitz (124) recognised three main subdivisions among the remaining Florideae, the Cryptonemiales, Gigartinales and Rhodymeniales. These are all distinguished not only by the origin of the gonimoblasts from special auxiliary cells but also by possession of tetrasporangia, for the most part borne on distinct plants. It was first established by Yamanouchi (167) in *Polysiphonia* that in these Florideae the postfertilisation nuclear divisions in the zygote are not meiotic, that the carpospores are diploid, that this is also true of the vegetative cells of the plants bearing tetrasporangia, and that the reduction divisions ensue during formation of the four spores in the latter. Similar features were demonstrated in *Martensia* (140), *Griffithsia* (73, 83) and *Delesseria* (141), while Lewis (84) by direct cultures established the alternation between haploid sexual plants producing diploid carpospores and diploid asexual plants producing haploid tetraspores. Since then this kind of life-cycle, styled diplobiontic by Svedelius (143, *43*), has been established for a considerable number of other genera belonging to the orders above mentioned.

The broad basis of classification among these diplobiontic Florideae still follows the lines laid down by Schmitz and depends essentially on the features of the reproductive process. Recent writers, following Kylin (76, *113;* 77, *90;* 81), distinguish Gelidiales, Cryptonemiales, Gigartinales, Rhodymeniales and Ceramiales. The order Ceramiales was first established by Oltmanns (90, *683*). It includes the majority of Schmitz's Rhodymeniales and is distinguished from the remnant, still grouped as Rhodymeniales, by the invariable uniaxial construction and by the fact that auxiliary cells are cut off from special mother-cells only after fertilisation. The Rhodymeniales in their present delimitation include multiaxial forms in which the auxiliary cells of the procarps, although cut off before fertilisation, do not attain full differentiation until that process has occurred. A further difference emphasised by Fritsch (42, *400*) is that in Rhodymeniales the germinating spore always divides by a number of walls perpendicular to the substratum to form an obscure prostrate system, prior to production of the erect growth, while in Ceramiales the first dividing walls in the spore are parallel to the substratum and growth is erect from the first.

The Gelidiaceae (*Gelidium, Pterocladia, etc.*), although possessing distinct tetrasporic individuals, resemble the less specialised Nemalionales, among which they were classed by Schmitz (124), and differ from other diplobiontic forms in the fact that the gonimoblasts arise directly from the carpogonia and that there are no auxiliary cells. There is reason to suspect a diplobiontic life-cycle, although this has not yet been cytologically established. Kylin's (75, *132;* 76, *24*) reference of these few genera to a separate order is, however, justified also on other grounds, *viz.*, the origin of the fruit from an aggregate of procarps (compound procarp of Kylin (76, *27*)). Certain Nemalionales (a few species of *Acrochaetium, Galaxaura*) bear tetrasporangia on distinct individuals, and it is within the realms of possibility that further study may necessitate their removal to the diplobiontic Florideae, but since tetrasporangia in various other species of *Acrochaetium* occur on sexual individuals, commonly together with monosporangia (neutral sporangia (135, *115*)), it is perhaps more probable that these tetrasporangia of Nemalionales merely represent monosporangia with divided contents and that they are not the seat of a reduction division (43, *550*).

Whilst Nemalionales, Gelidiales, Rhodymeniales and Ceramiales constitute well defined groups, this is scarcely true of the other two orders at present recognised, the Cryptonemiales and Gigartinales, which include a large number of common Florideae. The only essential difference is that in Gigartinales (79, *72*) the auxiliary cells are intercalary cells of the ordinary threads composing the vegetative system, while in Cryptonemiales the auxiliary cells are situated in special accessory branches. Although this is a taxonomic distinction easily recognised, it can scarcely be supposed to afford the basis for a natural classification. Both orders include families (*e.g.*, Grateloupiaceae and Dumontiaceae among Cryptonemiales, Furcellariaceae and Solieriaceae among Gigartinales) in which carpogonia and auxiliary cells are produced on distinct branch-systems, often remote from one another, and others (*e.g.*, Gloeosiphoniaceae and Callymeniaceae among Cryptonemiales, Rhodophyllidaceae and Gigartinaceae among Gigartinales) in which they are approximated to form more or less clearly defined procarps; in the former there is a development of often elongate connecting threads by means of which the products of division of the diploid zygote nucleus are transferred to the auxiliary cells. Such differences will perhaps provide a more satisfactory basis for classification of these Florideae into two or more orders. The nemathecial habit (including the corallinaceous conceptacle) might afford another character that could be deemed to be of ordinal rank. At one time Kylin (76, *121;* 77, *99*) recognised a further order, the Nemastomales (including Rhodophyllidaceae, Sphaerococcaceae, Nemastomaceae), but this was subsequently (79) merged in the Gigartinales, when the basis of distinction was found to be unsatisfactory.

In the two orders Cryptonemiales and Gigartinales Kylin recognises numerous families[10], distinguished *inter alia* by the features mentioned above, by the vegetative structure (uni- and multiaxial families), and by the mode of arrangement of the tetraspores (cruciate or zonate). The Rhodymeniales (78) are at present classed into Champiaceae and Rhodymeniaceae, of which the latter are not very clearly defined and possibly heterogeneous. The many diverse genera of Ceramiales have long been grouped in the three families Ceramiaceae, Delesseriaceae and Rhodomelaceae. Recently Rosenberg (115) has segregated from the last a fourth family, the Dasyaceae (*Dasya, Dasyopsis, Heterosiphonia*), distinguished largely by the sympodial construction of the vegetative system. The astounding diversity in vegetative organisation, despite a remarkable degree of uniformity in the structure of the reproductive organs and in the post-fertilisation events, suggests that the Ceramiales are a long-established group, the members of which have undergone pronounced evolutionary developments in the vegetative system in comparatively recent times.

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[10] The more important Cryptonemiales are: Gloeosiphoniaceae, Callymeniaceae, Grateloupiaceae, Dumontiaceae, Cruoriaceae, Rhizophyllidaceae, Squamariaceae and Corallinaceae; Gigartinales: Nemastomaceae, Furcellariaceae, Solieriaceae, Rhabdoniaceae, Rhodophyllidaceae, Hypneaceae, Plocamiaceae, Sphaerococcaceae, Gracilariaceae, Phyllophoraceae and Gigartinaceae.

CLASSIFICATION OF THE MYXOPHYCEAE

(Cyanophyceae, Schizophyceae, Phycochromophyceae)

The broad classification of blue-green algae adopted by Kirchner (62) in the first edition of the "Natürliche Pflanzenfamilien" and in part based on that of Thuret (151), was universally accepted during the earlier part of this century (159, 161). Kirchner subdivided the Schizophyceae, as they were called in the "Natürliche Pflanzenfamilien", into Coccogoneae with the families Chroococcaceae and Chamaesiphonaceae, and Hormogoneae (Hormogoneales of later authors) including the more definitely filamentous members of the class. The latter were grouped into Psilonemateae (with Oscillatoriaceae, Nostocaceae, Scytonemataceae and Stigonemataceae) and Trichophoreae (with Rivulariaceae and Camptotrichaceae), according as hairs terminating the trichomes were absent or present, but this distinction, which lays stress on a minor character, has rightly met with little favour. Setchell and Gardner (130) subdivided the Hormogoneales into Homocystineae and Heterocystineae, the former including only the Oscillatoriaceae, and this grouping is followed by G. M. Smith (135). It should be noted, however, that by no means all Heterocystineae possess heterocysts.

Increasing knowledge of the genera classed in the Chamaesiphonaceae led to recognition of more fundamental differences from Chroococcaceae than could be adequately expressed by inclusion of these two families in a common group, and so from 1925 (49, 162) we find the three orders Chroococcales, Chamaesiphonales and Hormogoneales figuring in the classification of Myxophyceae (*cf.* also 164).

Geitler's (49) detailed consideration of Myxophyceae, in preparation for his treatment of the class in Rabenhorst's "Kryptogamenflora" (50), clarified the classification within these three orders. As a result of his own contributions, especially those relating to Chamaesiphonales (48), and by bringing to the front a considerable number of tropical Hormogoneales described earlier by Borzi, Geitler demonstrated an appreciably wider range in morphological construction among blue-green algae than had been recognised in previous taxonomic works. Among Chroococcales he distinguished the largely palmelloid Chroococcaceae (*Chroococcus, Gloeocapsa, Aphanocapsa, etc.*) from a number of colonial forms showing something of a filamentous tendency, the Entophysalidaceae (49, *235*), including amongst others the important marine lithophytes *Entophysalis* and *Platoma.* To Geitler we also owe recognition of several different developmental series among Chamaesiphonales, as exemplified by the distinction, as separate families of the order, of the Dermocarpaceae, Chamaesiphonaceae and Pleurocapsaceae.

The heterogeneity of the group of forms classed in the Chamaesiphonales has led the writer (44, 46) to separate the Pleurocapsaceae and Hyellaceae as Pleurocapsales from the series of essentially unicellular epiphytes included in Dermocarpaceae and Chamaesiphonaceae, for which the old order Chamaesiphonales is retained. The Chamaesiphonales, in this more limited sense, are probably a branch-line from the Chroococcales, since certain species of *Dermocarpa* are closely related to *Chroococcus* and its immediate allies (44, *136*) ; the most important difference lies in the epiphytic habit and consequent polarity. Forms allied to *Dermocarpa* in which retention and uniseriate production of endospores has become the rule, have developed a pseudo-filamentous habit (*Stichosiphon* and ? *Endonema* (101)). On the other hand, there is every reason to believe that the characteristic exospore-formation of *Chamaesiphon* is derived from the process of endospore-formation seen in certain species of *Dermocarpa* (50, *416*).

The commoner members of the Pleurocapsaceae (*Radaisia, Pleurocapsa, Oncobyrsa*) are filamentous forms and, moreover, are typical examples of that variant of the heterotrichous habit that results in the formation of compact crusts. They are in fact a marked vegetative parallel to the Myrionemataceae among Phaeophyceae and to the Cruoriaceae and Squamariaceae *inter alia* among Rhodophyceae. The former inclusion of Pleurocapsaceae among Chamaesiphonales rested essentially on joint possession of endospores. These reproductive bodies, however, occur also in diverse Chroococcales and in *Herpyzonema* among Hormogoneales (158, *36*). Their pronounced filamentous character and heterotrichous habit separate the Pleurocapsales sharply from the Chamaesiphonales, and it may be doubted that there is any close affinity between them. Certain Pleurocapsaceae (*Xenococcus, Chroococcopsis*) exhibit a more or less pronounced tendency towards loss of the filamentous habit (*cf.* Chaetosphaeridiaceae among Chaetophorales).

The Hyellaceae (included in Pleurocapsaceae by Geitler, (50)) comprise a series of mainly lime-boring forms, of which *Hyella* has long been known. In recent years the family has been enriched by the description by Ercegović (29, 30), from the coasts of Dalmatia, of a number of other endolithic genera (*Scopulonema, Dalmatella, etc.*) which appear to be widespread in the Mediterranean area (4, *55;* 35). Although their generic status has been disputed (88), they indicate the existence of a considerable diversity in differentiation, especially of the endolithic system. The Hyellaceae are parallel, in both mode of occurrence and vegetative organisation, to the Gomontieae among Chaetophorales, and, like the latter, are characterised by the fact that the penetrating threads arise from the under side of the prostrate system, which inhabits the surface or surface layers of the substratum.

In his preliminary review Geitler (49, *252; cf.* also *27*) distinguished two series of Hormogoneales, the Nostocales and Stigonematales. The former included all the Hormogoneae of Thuret, the latter the forms grouped by Kirchner in Stigonemataceae, now classed in a number of different families and much enriched by adoption of Borzi's genera referred to above. This classification into Nostocales and Stigonematales was not maintained in Geitler's later work (50, *105*), although it has been resuscitated by the writer (44, 46), partly on the basis of other considerations.

The Stigonematales are distinguished from other heterocystous Hormogoneae not only by the abundant occurrence of true branching but also by their heterotrichous habit. Moreover, they usually possess definite pit-connections between the cells, and the threads show a considerable tendency towards apical growth. Four or more families are readily distinguished (50, *457;* 44, *142*). The heterotrichy is clearly differentiated in the Pulvinulariaceae, Capsosiraceae and Stigonemataceae, but is lacking in the specialised family Nostochopsidaceae (*Nostochopsis, Mastigocoleus*), distinguished by the frequent position of the heterocysts at the ends of short laterals. The least specialised members are comprised in the Pulvinulariaceae and Capsosiraceae, among which *Pulvinularia* and

Stauromatonema, respectively, exemplify a crust-development, analogous to that seen in many Pleurocapsaceae, while *Hyphomorpha* (Pulvinulariaceae) develops a prostrate system only and constitutes a parallel to similar forms in the heterotrichous groups of other classes of algae. A greater specialisation is evident among many of the less advanced Stigonemataceae (*Hapalosiphon, Fischerella, Westiella*) where the erect threads of the heterotrichous filaments are commonly little branched, usually differ from the prostrate ones in their narrower and longer cells and alone produce hormogonia, whilst the akinetes found in several genera are formed by the cells of the prostrate threads. The multiseriate character of the trichomes that characterises most species of *Stigonema* is foreshadowed in *Fischerella.*

Classification of the Nostocales remains much as it was in Kirchner's day. No satisfactory basis has so far been found for a further subdivision of the families Oscillatoriaceae, Nostocaceae, Scytonemataceae and Rivulariaceae, which are on the whole clearly defined and still retain much the same limits as were given them by the older systematists. The Camptotrichaceae of West (163, *268*) have, however, been suppressed; the rare non-heterocystous *Hammatoidea,* with trichomes tapering at each end, is now included in Rivulariaceae (49, *269*), while *Camptothrix* itself remains a genus *incertae sedis.*

The three genera *Aulosira, Microchaete* and *Hormothamnion,* grouped in a subdivision of Nostocaceae by Kirchner (62, *76*), have been referred to a separate family (Microchaetaceae) by Geitler (50, *664*), although the family appears quite heterogeneous and is of doubtful value. The only feature that *Aulosira* has in common with *Microchaete* is the firm, well defined sheath around the trichomes; in other respects it is much like *Anabaena. Microchaete,* in its possession of basal heterocysts, approximates to *Cylindrospermum,* while the marine *Hormothamnion* represents an altogether distinctive type amongst Nostocaceae.

A peculiar line of development among Scytonemataceae is that comprising *Brachytrichia* and a few other little-known genera (*Kyrtuthrix* (29); *Herpyzonema* (158)). With increasing knowledge these may provide the basis for an additional family of Nostocales. The Brachytrichieae are essentially characterised by a modification of that mode of origin of geminate false branches involving a process of loop-formation which has been observed in certain kinds of *Scytonemas*. In the former, however, the loops usually remain intact (28; 49, *217*) and there is a tendency for one arm of the loop to continue growth so that it later appears at the apex of the V-shaped lower portion. A similar method of branching is sometimes found in the hot-spring alga *Mastigocladus*, and, for this reason, Geitler (49, *263;* 50, *553*) established a family Mastigocladaceae to include this genus, as well as the Brachytrichieae. *Mastigocladus*, however, has very little in common in other respects with the Brachytrichieae and in certain stages shows true branching and a marked resemblance in habit to *Hapalosiphon* (34, *456;* 36, *179*). For the present it is best referred to Stigonemataceae.

CLASSIFICATION OF DIATOMS

(Bacillariales, Bacillariophyceae)

The present-day classification of this highly specialised group still follows essentially that of Schütt (127) in the first edition of the "Natürliche Pflanzenfamilien". He distinguished the two main subdivisions, Centricae and Pennatae, on the basis of the general symmetry relations of the cells, and these have since been recognised as independent orders (57; Centrales and Pennales of Karsten (60)). The Centrales are classed in four families, according as the cells are shortly cylindrical or discoid (Discaceae, *e.g.*, *Cyclotella, Melosira*), elongate-cylindrical with numerous intercalary bands (Soleniaceae, *e.g.*, *Corethron, Rhizosolenia*), box-shaped with valves having two or more poles (Biddulphiaceae, *e.g.*, *Chaetoceras, Isthmia, Triceratium*), or naviculoid but with radially arranged markings (Rutilariaceae with *Rutilaria*).

Schütt recognised four series of pennate diatoms (Fragilarioideae, Achnanthoideae, Naviculoideae, Surirelloideae). Karsten's (60) and Hustedt's (57) more recent schemes introduce a greater degree of subdivision which, like that of Schütt, is based essentially on the presence or absence of a raphe in the valves, and its mode of differentiation. They distinguish four main groups of Pennales, the Araphideae (*Fragilaria, Synedra, etc.*) in which the valves possess only a pseudoraphe, the Raphidioideae (*Eunotia, Peronia*) with a raphe limited to a short extension from the polar nodules, the Monoraphideae (*Achnanthes, Cocconeis, Rhoicosphenia*) with a fully developed raphe on one valve only, and the Biraphideae with a raphe on both valves. The Araphideae (Schütt's Fragilarioideae) are probably an artificial group, in some members of which the absence of the raphe may be primitive, whilst in others (*e.g.*, *Synedra*) it has possibly been secondarily lost, perhaps in adaptation to a planktonic existence. The status of the Raphidioideae is uncertain, but it is perhaps more probable that they are Monoraphideae (*Peronia*) and Biraphideae (*Eunotia*) in which the raphe is in course of reduction.

The Biraphideae include a large diversity of genera which are grouped as Naviculoideae, Epithemioideae, Nitzschioideae and Surirelloideae. The Naviculoideae (*Navicula, Pinnularia, Gomphonema, etc.*) are distinguished from the Epithemioideae (*Denticula, Epithemia, etc.*) by the position and nature of the raphe. In the former it occupies the apical (sagittal) axis and consists in each valve of two longitudinal clefts which are linked in the region of the central nodule. In the Epithemioideae, on the other hand, the raphe lies outside the apical axis, sometimes on an eccentric keel, and takes the form of a canal running longitudinally through the membrane of the valve. A similar canal-raphe is met with in the Nitzschioideae (*Nitzschia, Hantzschia, etc.*), but here it is always situated in a keel which is often eccentric; the raphe is here provided with the characteristic carinal dots, representing apertures on the inner surface of the raphe or the strips of membrane intervening between these apertures. In the Surirelloideae (*Surirella, Cymatopleura, etc.*), lastly, the canal-raphe occupies two lateral wings on the valves and is connected by obvious canals with the protoplast.

The relation of the two main orders of diatoms to one another remains as obscure as ever, although the suggestion of a possible distinct origin (92, *194;* 108) does not at present find much favour. As our knowledge of Centrales and Pennales increases, the contrasts between the two series become more marked. Karsten (60, *182*) was of the opinion that production of auxospores by sexual fusion in the majority of the Pennales resulted from the acquisition of motility by the latter, since the scanty data relating to Araphideae (*cf.* 41, *620*) suggest that auxospore formation may here take place in a manner similar to that observed in certain Centrales. Karsten believed that the Tabellariaceae were allied to some of the Centrales by way of such forms as *Terpsinoë* which possess naviculoid valves provided with numerous transverse septa. Geitler's (51) recent demonstration that in *Synedra ulna* auxospores are formed by conjugation of gametes from distinct individuals, not only necessitates reexamination of other Araphideae by modern methods, but suggests that some of them at least are closely related to Pennales provided with a raphe. There is in fact no adequate evidence to warrant the belief that the Araphideae are primitive among Pennales; some, and perhaps all of them, may equally well be reduced forms.

It may be doubted that, with the data at present available, it is justifiable to regard Centrales and Pennales as anything more than possible divergent lines of development from some common remote ancestry. That they are derived from a flagellate source, as many believe, because of the occurrence of swarmers in Centrales and the occasional observation of contractile vacuoles in Pennales (51, *555;* 106), remains to be more clearly established. Until more is known of the methods of reproduction of Centrales and of the Araphideae it is not profitable to discuss the matter further.

INTERRELATIONSHIPS OF THE VARIOUS ALGAL CLASSES

Pascher (95, 104) classes the algae as a whole as follows:—

I. Chrysophyta.
    A. Chrysophyceae.
    B. Diatomeae (Bacillariales of other authors).
    C. Heterokontae (Xanthophyceae of this article).
II. Phaeophyta (Phaeophyceae).
III. Pyrrophyta.
    A. Cryptophyceae.
    B. Desmokontae.
    C. Dinophyceae.
IV. Euglenophyta (Euglenineae).
V. Chlorophyta.
    A. Chlorophyceae.
    B. Conjugatae.
VI. Charophyta (Characeae).
VII. Rhodophyta (Bangiineae and Floridineae).
VIII. Cyanophyta (Myxophyceae).

Each of these main groups is regarded as having the same status as Bryophyta and other so-called "higher" groups, a point of view which it is not the province of this article to discuss. Smith (136) follows this grouping of Pascher.

The combination of Chrysophyceae, Xanthophyceae and diatoms in a common group, Chrysophyta, was suggested by Pascher in 1914 (95) and more fully substantiated in 1921 (97). He bases his view of the affinity between the three classes on the possession of a number of common characteristics; (*a*) preponderance of yellow or brown carotenoid pigments in the chromatophores, (*b*) absence of starch and occurrence of oil as a frequent photosynthetic product, (*c*) presence of leucosin, characteristic of Chrysophyceae, in certain diatoms (68) and Xanthophyceae (102), (*d*) frequent deposition of silica in the cell membranes (mainly in the cysts of Xanthophyceae and Chrysophyceae), (*e*) bipartite nature of the cell wall seen in the diatom frustule, in the vegetative cells and resting spores of diverse Xanthophyceae, and in the cysts of Chrysophyceae, (*f*) presence of envelopes composed of numerous pieces in Xanthophyceae (*Tribonema, Ophiocytium*), Chrysophyceae (*Dinobryon*) and diatoms (intercalary bands), (*g*) similarity between the cysts of Chrysophyceae and the endogenous cysts of the diatom *Chaetoceras,* (*h*) occurrence of endogenous cysts also in certain Xanthophyceae (*Chloromeson* (102, *405*)), and (*i*) presumed flagellate ancestry of diatoms. There appears to be substantial evidence in favour of close affinity between the flagellate members of Xanthophyceae (*Chloromeson, etc.*) and Chrysophyceae (especially the Ochromonadeae).

Diatoms are assumed to have the same status among Chrysophyta as the Conjugales have among Chlorophyta, but it must be emphasised that they stand far more isolated than do the Conjugales among Chlorophyta. The Pennales at least are diploid forms, with a highly derived sexual process, whereas the very scanty data we have in relation to sexuality in Xanthophyceae and Chrysophyceae point to their being haploid forms. Despite obvious similarities between the three classes in respect of membrane-structure, which may be homoplastic, there is nothing even approaching the astounding complexity of the wall attained in both groups of diatoms. The wide gap between the latter and the members of the other two classes renders their classification in one common group very problematic.

In the Pyrrophyta, Pascher (99, *51*) regards the Desmomonadaceae as comprising the most primitive forms, and he suggests a derivation of the Cryptomonadales from them by development of a dorsiventral organisation and of the longitudinal furrows which are so evident in the cells of *Cryptomonas,* for instance. In *Pleromonas* and *Haplodinium* the cells exhibit an apical incision which is regarded as the precursor of the furrows typical of Cryptomonadales, but the significance of these furrows is unknown and it may be that too much stress is being placed on this character. The Desmomonadaceae, however, show appreciable resemblances to Cryptomonadales in general organisation, in the presence of two dissimilar band-shaped flagella, in pigmentation, and in the possession of solid photosynthetic products.

A relationship between Dinoflagellata and Cryptomonadales was accepted by Klebs (64) and is also supported by Lindemann (85). In particular the Nephroselmidaceae (*Protochrysis, Nephroselmis*) among the latter, where the flagella are attached laterally and the furrows run more or less transversely, invite comparison with such a genus as *Hemidinium* among Dinoflagellata. The Nephroselmidaceae demonstrate that in the evolution of the Cryptophyceae there has been a shifting of flagella and furrow to the ventral surface, and such transposition probably also occurred in the evolution of the Dinoflagellata. The varied position of the flagellar apertures and of the furrow system on the ventral surface among Gymnodinioideae at least shows that shifting must often have occurred during evolution within the order Dinoflagellata itself. The exact origin of the type of cellular organisation typical of the latter is not clear, however, and there is no satisfactory basis for a direct connection with forms like the Desmomonadaceae. The undoubted resemblances between Cryptomonadales, Desmomonadales and Dinoflagellata are not, in the present state of our knowledge, of such a nature as to render homoplasy out of the question, and their classification in a common group is probably premature. On the other hand, the Dinophysiales among Desmokontae show an appreciable degree of approximation to some of the armoured Dinoflagellata, and an origin of Desmokontae and Dinokontae from a common stock appears highly probable.

Several authorities have hesitated to abandon altogether the former classification of the members of Xanthophyceae among the Chlorophyceae. Thus, West (161, *153*) included the Heterokontae as a division of Chlorophyceae and Printz (113) does the same.

All evidence, however, indicates that Xanthophyceae and Chlorophyceae are perfectly distinct evolutionary series. Steinecke's (138) interpretation of the former as a reduction-series, commencing with the filamentous forms which are supposed to be derived from *Microspora,* has no real basis in fact. Chodat's (21) inclusion of Heterokontae, Peridinieae, Bacillariales and Euglenineae among Phaeophyceae ignored many securely established facts and requires no serious consideration. No justification can be found for the reference of Peridinieae, Bacillarieae and Conjugatae to a common group Zygophyta by Wettstein (164), since these three series have no features in common with one another.

It may be doubted that any real progress is achieved by endeavouring to bring together any of the eleven classes distinguished by the author (41). The time is certainly not yet ripe for phylogenetic speculations like those of Steinecke (137) and Zimmermann (168), and, unless the fossil record furnishes data of a kind different from those it at present provides, it may be questioned whether such speculations will ever fulfill a useful purpose.

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CONTROL OF NOXIOUS PLANTS[1]

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INTRODUCTION

This discussion centers around those plants which are considered to be weeds. A weed, according to one authority (137), is a plant growing where it is desired that something else shall grow; according to another (294), it is "A plant that does more harm than good and has the habit of intruding where not wanted". A noxious plant is a weed that is especially undesirable because of certain attributes of it which prevent associated crop plants from attaining normal productivity. These undesirable traits may be of several types. The principal ones are persistence and vigor of growth, poisonous properties of herbage or fruits, and possession of spines or thorns injurious to animals. Other characteristics for a plant being classed as noxious may be advanced, but those listed include most weedy plants.

Few people realize the extreme seriousness of weeds. Too many take weedy plants for granted as necessary evils that we must endure, and because of their universality do not appreciate the damages done. The United States Chamber of Commerce (61) estimates the annual losses from weeds as compared with the more spectacular livestock diseases and insect pests as follows:

Diseases and plant poisoning of live stock..	$ 250,000,000
Insect pests	1,000,000,000
Weedy plants	3,000,000,000

In other words, weeds occasion greater losses than the other two groups of pests combined. However, the great amounts spent in

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combatting animal diseases or insects by far exceed the funds expended on weed control research. It would appear that the principal reason for this anomaly lies in a lack of appreciation of the seriousness of the weed problem.

Some weedy plants are poisonous to animals which consume them because they either contain poisonous compounds or produce poisons in the animal's digestive system. Most such losses occur in the western states where poisonous plants are more abundant than in the East. Injury of often as much as 8% of Colorado's livestock results from the effects of more than 100 species of such plants (118). Significant publications on poisonous plants are indicated in citations Nos. 70, 135, 145, 193, 239, 241, 280, 325, 326, 359, 363, and 371.

Many common weeds possess sharp structures as awns, thorns or barbs which may cause mechanical injury to livestock (197, 282, 362). In certain areas of the West, sheep may have such sore mouths as a result of them that they are unable to eat. Frequently eye wounds result in partial blindness which prevents their finding feed. Certain types of needle grasses may become imbedded in the flesh of sheep, and this materially lowers the quality of the carcass. A few of the more common weeds producing mechanical injuries are wild oats (*Avena fatua* L.), wild barley (*Hordeum jubatum* L.), puncture vine (*Tribulus terrestris* L.), sandbur (*Cenchrus pauciflorus* Benth.) and species of *Xanthium, Cirsium, Centaurea, Aristida* and *Stipa.*

Some weedy plants impart undesirable flavors and odors to the dairy products of cows which eat them (159), and millions of dollars are thus lost each year. The more common weeds producing bad flavors are species of *Allium,* including wild onion, garlic and leek. The Frenchweed plant (*Thlaspi arvense* L.) produces a similar flavor. Other weeds which impart undesirable flavors to milk are pepper grasses, wild carrot, ragweeds, marsh elder, smartweeds, mustards and bitterweed (*Helenium tenuifolium* Nutt.). Walker (386) found that removal of dairy cows at least three hours before milking from pastures containing milk-tainting weeds prevented contamination.

In some sections of the East wild garlic grows in wheat fields and the bulblets are harvested along with the wheat. Unless the bulblets are separated the garlic imparts a flavor to the flour, thus reducing its market value.

Poison ivy (*Rhus toxicodendron* L.) and poison sumac (*Rhus Vernix* L.) are examples of weedy plants which may cause a severe itching rash on the skin of most persons. Some people are resistant or immune; others are very susceptible. Much time is lost each year by those who are infected, and in some cases complications result in death. Grand and Hansen (147) and Yeager and Calahan (406) give control measures.

Hayfever causes losses of millions of dollars each year and makes life somewhat miserable for thousands of citizens. Probably the most common cause is the pollen of the common ragweed (*Ambrosia elatior* L.). Few areas of the country are free from its pollen and these sections become havens of refuge during late summer when the plants are in bloom.

Weeds harbor numerous parasites and diseases. Sallans (324) notes the relationship between weeds in cereals and development of root-rots, while Wallace and Murphy (387) report on certain weeds which harbor sugar-beet diseases. Some weeds appear to be carriers of leaf-roll and rugose mosaic of potato (119), and the cotton root-rot fungus, *Phymatotrichum omnivorum* (Sh.) Dug., lives on many species (318). Piemeisel (293) reports on the relationship between weedy plants of Idaho and the curly top disease.

Investigating the probable effect of weeds on soil fertility, Snidor (351) analyzed 15 common species in central and northern Illinois. The percentage of nitrogen, phosphorus, potash, calcium, magnesium, iron and manganese compared favorably with that of various crop plants such as alfalfa, clover, timothy, red top, corn stalks and wheat straw. Weeds feed heavily and utilize fertility which would otherwise be available for competing crop plants. Concerning their effect on the nitrate content of soils, the upper three feet, where weeds were permitted to grow, contained 81.6 pounds of nitrates, while a comparable area with a three-inch mulch contained 413.3 pounds of nitrates to the acre (64).

Practically every State has laws regulating the sale of crop seeds and limiting the sale of those containing mixtures of certain undesirable weed seeds. Aside from the reduced yields of the crop variety as a result of weed competition, the presence of the weed seeds greatly lowers the market value of the crop seed and thus imposes a double tax on the farmer. Rather (307) discusses some of the significant relationships between weeds and the marketing of farm seeds.

Plants commonly classed as weeds are not always harmful, however. They may do much good in providing cover to prevent erosion and through their growth may increase the available fertility of soil; tobacco, for instance, following certain species of weeds, is superior to that produced on bare fallow (237).

CHARACTERISTICS AND FOOD RESERVES OF WEEDY PLANTS

Before one can hope to effectively control undesirable weed plants, it is important that he understand the fundamental characteristics pertinent to each species. A very desirable plant under certain conditions may be undesirable if it becomes so well established that it effectively competes with desired species. Sweet clover persisting in a cultivated corn field may prove highly undesirable, and to all purposes it is a weed under these conditions. Numerous investigators have considered the physiology and anatomy of various weed plants with a view to learning principles which might direct their modes of attack in securing most efficient control. It seems desirable to review some of the fundamental work before turning to specific control measures.

Cole and Holch (71) discuss the root systems of 21 weeds in southeastern Nebraska. Kiltz (206) describes the root systems of 10 perennial weeds. Hardy (160) discusses the root systems of several weeds in relation to tillage. There is also a very complete study of the root development of weeds and crops in competition, in which Hannchen barley competed more successfully with wild oats and wild mustard than did Marquis wheat, due to the more rapid seminal root development of the barley (287–289). Norris (269) reports on an ecological study of weeds in eastern Nebraska. An excellent treatment of the anatomical development of *Lepidium Draba* (L.) is reported by Simonds (337). Kennedy and Crafts (200) discuss the application of physiological methods to weed control. In later work (201) the same authors describe the anatomy of field bindweed. Kirk and Pavylchenko (209) report that land infested with wild oats may produce new plants by vegetative regrowth following tillage shortly after the seedlings emerge. The vegetative reproduction depends upon the moisture of the soil and the degree of packing.

An important function of the thickened roots and rhizomes of perennial weeds is to store reserve food materials. While some of this reserve material may be used by the plant during the dormant period, by far most of it remains there until the critical period in spring when the plant must produce considerable new growth of leaves and stems before it can again synthesize all the food materials it needs. A portion of the reserve material is used to carry on the normal respiratory processes of the plant. Plants endowed with the capacity to store great reserves are equipped to survive under unfavorable environmental conditions. It is only logical to expect that these plants are very likely to be extremely persistent and thus frequently to fall in the category of weeds.

In a consideration of the reserves of a weed, one is concerned with the anatomy of the plant. In most cases, the root characteristics are of special significance, since the roots serve as the primary storage organs and thus play an important part in the ability of many species to persist as weeds. Field bindweed roots may penetrate the soil to a depth of 20 feet (21), and may yield a maximum acre production of 7,200 pounds (23). Total sugar and polysaccharide reserves in the roots were less than 600 pounds. The authors believe that the persistence of bindweed under fallow is due to the small quantity of reserves necessary for regeneration of new growth and to slow rate of removal of reserves from the roots at lower depths. Application of sodium chlorate resulted in an immediate drop in root reserves, suggesting a direct effect of the chemicals upon the respiratory rates or other processes in treated roots. Both fallow and chlorate treatments resulted in rapid depletion of polysaccharide reserves in the roots in the upper foot of soil.

In studies of bindweed reserves, total sugars reached a maximum of about 7% late in October with a minimum value in May (28, 29). Starches increased gradually, reaching a maximum in August or September, followed by a sharp decline coincident with the rapid increase in sugar percentage. Cultivation at two week intervals held the total sugar and starch content each to about 1%. Fall applications of sodium chlorate were more effective than early applications in reducing root reserves and controlling the weed.

In field bindweed studies in Kansas on the readily available carbohydrates at different dates of sampling, all carbohydrate fractions except the reducing sugar were lowest between April 15 and May 15. Total sugars were highest in the lateral roots and the first foot portion of the two types of vertical roots the first of November. In other root portions there was a tendency for the high point to be on or before July 15 with a maximum later in the season but prior to November 1 (128).

In very comprehensive trials in the control of field bindweed in western Kansas, the percentage of readily available carbohydrate reserves declined rapidly with continued cultivation until about July 1, after which it remained about the same. The total amount of living roots decreased slowly at first but more rapidly after July 1, and this decrease continued until all plants were dead. At the end of the season approximately 95% of the roots in the surface 18 inches of soil had died, with complete eradication usually by the middle of the second year. Following a single cultivation of untreated bindweed having a high level of root reserves, the percentage of carbohydrates decreased slowly until about four days after first emergence of the bindweed. After this, the decrease was more rapid and continued for a period of from 8 to 20 days after first emergence with an average of about 16 days. Following 16 days, as a rule, there was a rapid increase in reserves as the plant began building up a surplus. This work proves that bindweed may be eradicated just as quickly and with fewer cultivations by cultivating every two to three weeks as by continuous tillage (377). Results at Lamberton, Minnesota, were similar (402). In fact, at Lamberton continuous cultivation requires a somewhat longer period to secure complete eradication than where intervals of two weeks after emergence are followed.

Field bindweed appears to maintain itself when the moisture content of the upper two feet of the soil is below the wilting coefficient (20), and to produce an abundance of seed in seasons of high temperature and low rainfall (56).

Rapid movement of reserve materials out of the storage roots early in the season results in a reduction in dry weight parts at the time, followed by an increase in dry weight as the reserves are again stored in these organs. In data bearing this out, the underground storage organs of quack grass and Austrian field cress reached their maximum weight earlier in the season than did leafy spurge, sow thistle or Canada thistle. The author (11) attributed this to the greater leaf area of the quack grass and cress formed the previous season, which lived over winter and was ready to synthesize food as soon as growth started in the spring. He found sharp declines from late April through the first part of May in the percentages of total sugars and total readily available carbohydrates in the storage roots of each of the weeds with the exception of quack grass. The total readily available carbohydrates in the storage roots of leafy spurge and Austrian field cress were lowest for the season by the middle of May, at which time the plants were beginning to flower. Rapid storage of these materials occurred for a time and continued at more moderate rates to the end of the growing season.

Sow and Canada thistle, two weeds which start growth later in spring, declined rapidly at first in total readily available carbohydrates and continued at more moderate rates to the first part of July when the plants were in flower. Reserves accumulated from that time to the end of the season.

Rhizomes of quack grass were lower in both percentage and pounds per acre of total readily available carbohydrates in April at the time the first determinations were made than on any later sampling date, indicating depletion of reserves as a result of the winter season.

The readily available carbohydrates in the underground storage organs of each of the weeds except quack grass were low in relation to the total reserve carbohydrates in the middle of July.

Marked decreases in the percentages of true starch and increases in sugars in the underground storage organs of sow and Canada thistles and leafy spurge accompanied the lowering of temperatures in the fall. It appeared that similar changes did not occur in the underground storage organs of Austrian field cress and quack grass.

There were rapid declines in total organic nitrogen in the underground storage organs of sow thistle and Austrian field cress and less marked declines in those of Canada thistle, leafy spurge and quack grass during the early part of the season. These declines continued at a reduced rate until August, after which increases occurred.

Arny found the common form of storage sugar in underground parts of plants was the non-reducing sucrose or cane sugar. This is changed to simpler reducing forms before being utilized by plants. Thus the presence of reducing forms of sugar in any amount usually indicates activity in the translocation of reserves.

In sow thistle the percentage of reducing sugars increased during late April and the first part of May. Austrian field cress, Canada thistle and leafy spurge decreases were noted during the first part of the sampling period.

As the temperatures lowered in the fall, leafy spurge, sow thistle and Canada thistle showed a change in the carbohydrate reserves from starch to sugar. In neither quack grass nor Austrian field cress was there any indication of marked change from starch to sugar with the lower temperatures of fall.

The storage organs of sow and Canada thistle contained a lower percentage of hemicelluloses than the other three weeds. Hemicellulose reserves are relatively more stable than the other carbohydrate reserves and hence are used as the supply of the more labile materials is reduced. An increase of hemicelluloses during the early part and lowering during the latter part of the sampling period was the trend. In general, this was the opposite of what occurred for the more readily available carbohydrates.

With leafy spurge, Austrian field cress, sow thistle and Canada thistle the total and readily available carbohydrates reached low points at the same time the plants began to flower. Except in field cress, the total organic nitrogen was lower at this period. It would appear then that this should be a good time to begin eradication operations.

Under Minnesota conditions the weather is usually more favorable for eradication by tillage during July and August than either earlier or later. Considering all points, the first part of July appears to be a good time to initiate eradication of quack grass.

Cutting the tops of weeds near the soil surface at beginning bloom compels the plant to build a new set of aerial organs which involves additional drain on the reserves in the underground parts and has the further advantage of preventing seed formation.

Under Missouri conditions the weight of reserve storage organs of timothy and their nitrogen content was lowest in early spring and increased rapidly to early bloom and then less rapidly to maturity (382).

The percentage of total carbohydrate reserves in underground parts of Canada thistle was lowest during the latter part of June when the plants began to flower (315), and in winter studies of the activity of the roots and its relation to control methods, the author noted the development of buds upon the roots and of new horizontal roots upon the old storage organs (316). Ohio investigations

(392) on reserves in the roots of Canada thistle, calculated on the basis of green weights, seem to substantiate these findings. The data show that the nitrogen content of the roots tends to decrease to about July and then to increase again. Clipping the plants resulted in lowered food reserves in the roots.

Root reserves of white weed showed no material change in carbohydrate percentages until 14 to 16 days after cultivation, and biweekly cultivations were equally effective as weekly cultivations (30).

Sturkie (367) reports that cutting Johnson grass for hay in immature stages weakened the plants. Where he cut the plants but once a year when the plants were relatively mature and the seeds in the milk stage, there was a 40% greater development of rootstocks than where the plants were harvested continuously in the immature stages. The plants harvested when mature were much more vigorous and produced much more vegetation the following year.

Dexter (109) studied the winter hardiness of weeds as affected by fertilizers. He found that Canada thistle, downy brome grass and field bindweed became more hardy with the coming of cool weather. If photosynthesis were prevented in the fall, quack grass receiving nitrogenous fertilizer failed to harden while the unfertilized rhizomes did harden. Naturally the unhardened plants were more subject to winter injury. Later investigations (195) report that applications of nitrogen stimulated a vegetative response in more top growth. Continual removal of this top growth caused the plant to draw upon its organic reserves, resulting in carbohydrate starvation and death of most plants in about 24 weeks. Frequent defoliation prevented quack grass rhizome formation and destroyed the plants, even though they were fertilized (108), and rhizomes from plants fertilized with ammonium sulphate were more subject to drouth injury than rhizomes from untreated plants (110).

Dexter (111), studying the reserves of quack grass, recommends thorough tillage in the early part of the season to secure drying out and most effective destruction.

The reserves of several pasture weeds have been found to be lowest just before bud formation (146). This has an important relationship to the best time to destroy the weeds. In buckbrush, sumac, vervain and iron weed of Kansas, there also was a reduction of root reserves from spring to the time of budding or flowering, and then an increase to the close of the season (3). Cutting treatments begun when the reserves were low were more effective than when begun at other times.

CONTROLLING WEEDS

There are two general methods of approaching the weed problem—one is to work for outright eradication of the weed and the other is to keep it under such control that its harmfulness is greatly reduced. With certain very bad weeds the former method is to be desired and should be practiced wherever possible. In general, most weeds such as the more common annual species are held under control without actual eradication. In fact, their complete eradication would be so costly in most cases that it would not be economical to carry out the necessary practices. One prominent agriculturist has defined weed control as "good farming", meaning that a proper system of farming with operations well performed results in weed control.

Many general books have been written on the subject of weeds. Georgia (137) has prepared an excellent book which gives good illustrations as well as descriptions of plants and methods of control. While the book is somewhat old it is extremely useful. No weed library would be complete without Gray's Manual of Botany (149) and the splendid publications by Britton and Brown (52).

Robbins, Crafts and Raynor (313) and Muenscher (260) have published up-to-date books on weeds. Other general weed books have been written by Pammel (281), Pammel and King (283), Korsmo (214, 216) and Spencer (353).

A well-illustrated bulletin treats of representative Missouri weeds (115). Larson *et al.* (220, 221, 222) describe and illustrate several of the more common weeds. Cox (81), in a government bulletin, discusses weeds in general. Darlington *et al.* (102) describe 94 Michigan weeds and give methods of control.

Wilson and Larson (401) published a manual which deals with identification of the more common weeds. It is illustrated with numerous drawings which emphasize the characteristics making identification possible. In addition, the book treats of crop judging with emphasis on the significance of weed seed mixtures.

Muenscher (258) lists the weeds of New York by families. Gates (136) gives a short but concise treatment of methods of controlling various types, and a comprehensive discussion of the more important weedy plants of Kansas. Brenchley (46) treats of weeds in England. Robbins (311) gives methods of control used in central Europe. Several publications (25, 40, 44, 45, 60, 69, 78, 96, 99, 106, 113, 121, 151, 155, 194, 203, 207, 212, 215, 229, 246, 256, 275, 276, 277, 284, 285, 296, 306, 323, 340, 358, 364, 373, 383 and 400) deal with weeds in various other areas.

Control of noxious plants usually requires the use of one of two methods: cultural methods or chemical eradicants. Either may be used alone or both in combination. Cultural methods are considered to include rotation methods, growing of so-called smother crops, or any system of farm management which may aid in weed control. Use of chemicals involves application of certain toxic compounds capable of destroying the undesirable plants. Man has employed tillage methods for centuries. Extensive use of chemical eradicants has been confined to recent years. The proper evaluation of weed trials is important. Lynes (238) has shown how modern statistical methods may be applied to weed research.

Weed control is no longer a simple matter of maintaining good crop rotation or doing a thorough job of preparing a seed bed. With the great spread of especially vicious weedy plants, in many cases control revolves around specialized programs planned for particular species. For this reason it is logical that the published research be considered under more specific headings.

It is not practical to discuss the research with all weeds. It seems desirable rather to select certain phases of control work and to present the results secured from a number of investigations. The principles apply to species possessing similar characteristics.

Tillage Methods

As early as 1886, Sturtevant (370) showed that where weeds were permitted to grow, corn yielded 18.1 bushels per acre, while where the weeds were pulled and the land received no cultivation, the yield was 70.5 bushels. Elsewhere a six-year average yield of corn was 7.0 bushels per acre where weeds were allowed to grow, 53.3 bushels where the weeds were scraped with a hoe (403). Similar results have been reported by other authors (59, 67, 177, 204, 228, 373).

Pereira (291) reports on a series of cultivation tests with several row crops. He summarizes a number of British and American experiments on tillage which agree with his work in support of the theory that the principal purpose of cultivation is to control weeds.

In weed control, tillage may vary from the simple act of hand pulling the undesirable plants to the use of specialized machinery constructed expressly for destruction of weeds. The duckfoot cultivator is an example of such a specialized implement.

Hardy (161) does not favor deep plowing, as he believes that such a procedure merely postpones the difficulties. He recommends that annual weeds be given a light disking to cover the seeds in the fall. The following spring when the plants are about five inches tall they may be destroyed by shallow tillage. He believes the land should not be cultivated deeper than four inches in the control of annual weeds. The shallow tillage may be repeated five or six times during the summer season.

Shedd, Collins and Davidson (335), discussing weed control from the viewpoint of the engineer, state that the principal purpose of corn cultivation is to control weeds. The spring tooth weeder and rotary hoe were effective in destroying weed seedlings when the soil surface was lightly crusted by moderate rainfall. They did not prove as effective if the soil crust was heavy or was loose and dry. For a program of tillage they recommend six sweeps per row and rotary hoe shields for small corn. For the second cultivation a pair of disk hillers was used to throw the soil into the row. For the last cultivation similar treatment was followed, using two pairs of sweeps per row. The speed of the tractor cultivator should be between two and a half and four miles per hour.

Dieffenbach (112) describes weed machinery used in Utah and Idaho. Robbins suggests (312) control in orchards with both tillage and herbicides. Smith (345) discusses machinery suited to the control of field bindweed by tillage. Bioletti (34) at Davis, California, found that ordinary tillage would not control bindweed. By cutting the roots three inches below the surface every five days he was able to secure close to 100% kill in one season. Barnum (27), also working at Davis, California, was unable to control bindweed when the tillage intervals were two weeks. He recommended cultivating four to six inches deep about every five days. He reported that hogs may destroy bindweed by eating its roots. Wilson *et al.* (402) were able to greatly reduce the cost of eradicating field bindweed by tillage by extending the intervals between cultivations with a power operated duckfoot cultivator to two weeks after emergence of the new growth. Srb *et al.* (354), using a duckfoot cultivator and tractor, found that field bindweed in Nebraska could be eradicated at a cost of approximately $10.00 per acre.

Since special weed tools are costly and difficult to obtain, considerable work has been done to develop homemade implements. There are publications giving information on the construction of such machines (397, 402, 407). The Kansas State Board of Agriculture (8), in a discussion of field bindweed eradication, presents the results of Kansas control methods, as obtained on the farms of the State. Included are illustrations of methods of developing homemade implements.

Timmons (376) states that clean cultivation is the most dependable of all eradication methods. Best results were obtained when the bindweed was permitted to grow several days above ground after each cultivation with the duckfoot. Clean tillage costs ranged from $5 to $10 per acre. Frazier (127) recommends 14-day intervals between cultivations.

In southwestern Minnesota a thorough job of tillage spread over two years to eradicate field bindweed cost $12.70 per acre. This cost covered the period from 1936 to 1941 and included plowing once each season at a depth of five inches and ten cultivations each season with a duckfoot cultivator (402).

In other detailed experiments it required more than 70 cultivations over 2½ years to secure 100% kill of field bindweed on a 32-acre field that was cultivated as soon as the plants emerged (346).

General papers on bindweed control include (32, 122, 199, 205, 328, 400).

Canada thistle (*Cirsium arvense* (L.) Scop.) may be spread vegetatively by cuttings of horizontal or vertical roots and of upright subterranean stem shoots bearing nodes. Portions of root or of stem ⅛ to ¼ inch in diameter and ½ inch long produced new plants under favorable conditions (170).

Porter (295) advises plowing deep in July or August followed by fallow once a week until late fall to eradicate Canada thistle. The operations should be repeated the next year. A smother crop as millet, Sudan grass or sorghum may be planted in June. If the work is done properly, eradication should be complete within three years. Tillage on large areas and sodium chlorate on smaller infestations are also recommended as means of eradicating Canada thistle (399).

Quack grass (*Agropyron repens* (L.) Beauv.) is best adapted to the cooler sections of the United States, being well suited to the upper Mississippi Valley. Not infrequently perennial sow thistle (*Sonchus arvensis* L.) is found growing on the same farm, as the regions of adaptation for the two weeds are similar. Quack grass is more widely distributed than is sow thistle. Both species possess underground root structures which make it difficult to control them with ordinary tillage.

Arny (9) states that thorough tillage is necessary to eradicate quack grass. He recommended plowing in July, three to four inches deep; replowing not later than August 15, five to six inches deep; and again in November, six to seven inches deep. The disk was used between plowings to keep the quack grass from showing green above ground. Other authors give cultural methods for control of quack grass (384), recommend deep plowing (25–27 cm.) to destroy it (334), discuss the use of shallow tillage in the control of perennial weeds (290), outline the methods for controlling quack grass in South Dakota (189) and give information pertinent to the entire country (202).

Stoa *et al.* (365) reported spring and fall tillage as less effective than summer tillage in eradicating quack grass. Working at the Crookston Station, Dunham (117) recommends early spring and late fall plowing, with a thorough bare fallow to secure eradication of perennial sow thistle. In unpublished work in Minnesota, Arny was able to obtain 99.6% eradication of leafy spurge (*Euphorbia Esula* L.) in one year at a cost of about $12 per acre. Two years of fallow at a cost of $24.00 per acre were required to secure complete eradication.

Cultivating the land 30 times each year for two years destroyed leafy spurge, but cultivating corn four or five times in a season failed to eradicate it (18, 19). The same author describes the characteristics, habits and distribution of leafy spurge in Iowa. In another discussion of the characteristics and control of leafy spurge, sodium chlorate was best for small areas, and cultivation best on large sections (156, 157, 158). Tinline (381) gives methods of eradicating leafy spurge in Canada.

In studies of perennial peppergrass (*Lepidium Draba* L.) two seasons of thorough tillage followed by two years of check-rowed crops was the most economical and practical method of eradicating the weeds (187). Other workers outline methods of tillage for the control of white top (171, 172, 250, 319).

A year of cultivation of irrigated land infested with *Lepidium Draba,* followed by a very heavy seeding of grasses, clovers and alfalfa, resulted in crowding out of the weeds (125). Andrew (5), quoting Ewart, recommends summer fallow started with deep plowing, followed by a root crop as an effective means of controlling this weed.

One of the worst weeds of the South is Johnson grass (*Sorghum halepense* (L.) Pers.). It is to the South very much the same as quack grass is to the North. Deep plowing in June, July or August with removal and destruction of all roots and tops from each plowed furrow was effective. The land is plowed again in late November. The following year the process is repeated until eradication is complete (178).

Johnson grass is very bad in southern Indiana (226). Control was secured by plowing winter wheat land as soon as the crop was harvested with a disk or cultivator being used to keep the land black until fall. Wheat was seeded and the operations repeated a second year. Methods of controlling quack grass in New Mexico have been outlined in detail (278).

Nut grass was eradicated by plowing at intervals of three weeks during two successive growing seasons (348, 349). Areas likely to remain wet for long periods do not respond to cultivation. Exposure of nut-grass tubers to four days direct sunlight resulted in their destruction (347). Fromm (132) reported that simultaneous tillage and application of a 2 N thiocyanate solution proved equally as effective and cheaper in the control of nut grass than methods of tillage. One liter of 2 N calcium thiocyanate per square meter reduced the stand to 1% or less in 90 days.

Wild garlic does much damage in areas where adapted, and in addition to competing with crop plants, it lowers their market quality. General control is discussed by Skiver (343). Use of orchard heating oil for its destruction offers promise (13).

Brush-like plants such as brambles may be classed as weeds, since they interfere with the growth of desired plants. Comprehensive methods of control in pastures are available (43, 100). Effective control of brambles was obtained when sodium chlorate was applied about the time of fruiting (77). Renovation of pastures and its effect upon the control of weeds is closely related to brush control (134).

Mowing brush in July was the best time to subdue undersirable species (53). The most effective date of cutting was closely correlated with the time when the roots of the bushes contained the least amount of organic reserves (4).

Wild ferns are a serious menace in some sections. Through proper management they may be controlled in pasture lands of eastern United States (80).

While there are many other weedy plants of importance, all may be controlled by following the basic principles of clean cultivation. Prevention of growth ultimately exhausts the reserves of even the most vigorous weedy plant and may lead to its final destruction. The problem before the farmer is to utilize the best and most economical methods of control. In many cases these involve tillage alone, in others the use of chemicals, and in some instances a combination of tillage and chemical eradicants.

Brenchley and Warrington (48, 49, 50, 51) give a very complete report of the influence of crop, soil and methods of cultivation upon the relative abundance of viable seeds. In Sweden, harrowing to control weeds gave an average increase in grain yields of 8% and a second harrowing gave an additional increase of 3% (116).

Special Methods

A few special methods of control have been developed. Use of a flame, for instance, has been recommended as a successful and practical method of eradication (79), and the effects of ensiling are not to be overlooked (409). Under the latter category, seeds of rough pigweed, yellow foxtail, Johnson grass, smartweed, sunflower and cocklebur have been found to be destroyed by storage in sorghum and corn silage, while seeds of field bindweed, velvet weed, annual morning glory, giant ragweed and barn-yard grass were resistant (409). Of 29 varieties stored in alfalfa grass silage for periods of one week to five months, most of the seeds lost their viability but a few retained it even after storage. The weeds whose seeds were most resistant were lespedeza, field bindweed and dragonhead mint (405). Tildesley (375) destroyed the viability of 19 species by storage in silage juices which showed 1.5 to 2.0% of organic acids.

Any method of weed control must consider the animal as an important means of distribution, as it has been shown that not all weed seeds lose their viability in passing through the digestive tracts of farm animals (17, 211, 274). The proper composting of manure from animals consuming weed seeds is a recommended method of weed control. At the Rothamsted Station the cumulative effect of long-continued manuring appeared to be of secondary importance in relation to weed flora except in certain instances of serious deficiency, and the manures used did not seriously increase the weed population (388). One month of storage in cow manure and horse manure destroyed all seeds with the exception of velvet weed, bindweed, sweet clover and peppergrass (162). Field bindweed retained its viability after storage for four months in piles of chicken manure (366). Composting to control weeds relates to the entire subject of weed seeds in relation to their environment, and the effect of temperature variations on seed germination in the soil is important (1, 2, 33, 389). It must be remembered that not all composting is done well, and many weed seeds escape destruction, even though an effort is made to eliminate them by composting.

At Fort Hays, Kansas, an average of 20 tillage operations the first year killed 85% to 99% of the field bindweed plants (65). Other investigators proved that bindweed could be destroyed by shallow tillage only if the cultivations were made at frequent intervals (360). Control of bindweed in Scotland was effected by deep plowing and cultivation followed by harrowing to drag the roots into heaps (350). In Missouri the removal of trash, followed by 8-inch plowing about May 1 and then clean cultivation with a duckfoot cultivator every week to ten days until October 1 to 15, is a recommended practice (124). On land subject to erosion, rye may follow the clean tillage. Cultivation each eight to ten days was effective in eradicating bindweed, while tillage at intervals of two weeks with wheat seeded in the fall reduced the number of cultivations and permitted the growing of a cash crop (138). Other investigators have obtained comparable results (126, 205, 408). Control of field bindweed by repeated and gradually deepened plowing has shown promise (210), while Smith (345) has devised special weeder machines to expedite the fallowing of infested land.

Cropping Methods

As has been shown, any system of tillage involves added expense and constitutes a severe tax upon the farm. If it is possible to grow crops during eradication, one may greatly reduce his costs. It is well known that most plants, grown together, are in direct competition with one another. This competition may seriously affect crop yields. In western Kansas grain and forage yields of nine different crops were reduced from 20% to nearly 90% by field bindweed competition (377). Tall growing, broad-leaved plants exclude light from the shorter or slower growing plants. Some workers refer to certain types of crops as smother crops. No plant actually smothers another; a so-called smother crop is one that merely screens the sunlight from the lower plants.

Pavlychenko and Harrington (288) did an excellent piece of research on the competitive efficiency of weeds and cereal crops in showing that competitive efficiency was related to the ability of the plant to develop rapidly after germination. They write: "In competition supremacy may be attained by the species or variety which is able, by virtue of greater physiological activity and morphological adaptability, to utilize the environment more efficiently. Rate of growth may be the best manifestation of such efficiency. Readiness and uniformity of germination, if characteristic of a species or variety, may be of considerable importance. Similarly, facilities for the absorption of water and nutrients and for the assimilation of carbon dioxide may be important factors. These facilities involve all of the plant, including the underground portion". Similar work in Germany expressed viewpoints more or less in agreement (218, 219). To discover desirable types of plants capable of suppressing the undesirables is one of the objectives of weed research.

Research at the Lamberton, Minnesota, Weed Station has shown that sudan grass, millet, sorghum, soybean, alfalfa and brome grass are excellent competitors of field bindweed, provided a period of cultivation precedes seeding of the crop (355). Light values under different crops were determined with a Weston photo-electric cell, and correlations were found between the lower values and field competition, as evidenced by reduced vigor of the bindweed plants.

While both sunflowers and hemp have been credited with being good weed competitors, neither proved satisfactory in these trials, as it appeared that both species lost their lower leaves rapidly, thus permitting light infiltration.

Blackman and Templeman (38) concluded that in a year of normal rainfall competition between the crop and the weed is principally for nitrogen and light.

Using a photo-electric cell, a German worker studied the effect of various crops as shade plants to compete with weeds, and found rye was a better shade plant than wheat because the rye shade increased more rapidly (304). In work at the Lamberton Weed Farm in Minnesota it has been observed that winter wheat was more effective in competition with field bindweed than rye, since it maintained its shade later in the season. This is true only where winter wheat is well adapted.

Field bindweed was checked through a combination of tillage and the growing of rye (126). Other workers found alfalfa to be effective in checking the growth of bindweed (21, 328). Russian sunflowers reduced the stand about 90% (360).

Studies in Minnesota by Arny (10) have shown that Canada thistle may be eliminated through the growing of alfalfa. Thorough seedbed preparation is essential to permit establishment of the alfalfa crop. Following this, frequent cutting of the alfalfa does not permit the thistles to recover, and they may be eradicated within two to three years. Other workers have secured similar results (107). When the alfalfa was cut three times the year after seeding, no thistles were found the following or third year, with a good stand of alfalfa averaging 4.5 tons of hay per acre.

In Saskatchewan, crested wheat grass in three seasons completely smothered thick stands of perennial sow thistle and toad flax (286). In four seasons it eliminated Canada thistle and effectively controlled quack grass, leafy spurge, poverty weed, field bindweed and hoary cress. In Minnesota (402) it has been possible to control field bindweed by growing alfalfa and cultivating the latter until the middle of summer. A season of tillage with a duckfoot cultivator followed by rye or winter wheat where adapted has also proved successful in eliminating bindweed under Minnesota conditions, and many farmers have used the rye or winter wheat program in ridding their farms of this weed.

Use of heavier rates of seeding and fertilizers drilled with the seed to increase competition have checked annual weeds in cereals (142, 143). Nitrogenous fertilizers were used to increase competition between crop plants and vetch (303). There is some evidence to indicate a direct competitive effect of the growing plant. In a series of pot studies, rye or winter wheat grown with the weeds *Anthemis arvensis* L. and *Matricaria inodora* L. under certain conditions inhibited germination and development of the weeds (305). Rye exerted a more depressing effect than wheat, believed to be due in part to an antagonistic factor.

Grazing animals may be considered in relation to cropping practices. If livestock can be grazed effectively one may eliminate the weedy plants and at the same time gain a fair return from the forage provided. Various claims have been made regarding different classes of livestock used in this way. Sheep probably have proved more generally effective because of their willingness to eat herbage rejected by most animals and their inclination to graze closely, thus preventing plant recovery. At the Lamberton, Minnesota, Weed Station a period of cultivation followed by rye or winter wheat grazed heavily in the spring until the crop was consumed eliminated field bindweed within two to three seasons. Following death of the pastured grain, the land is plowed and either of two plans may be followed. The area may be cultivated until fall when fall grain is again seeded or sudan grass may be planted as soon as a good seedbed can be prepared. The latter method reduces labor costs and provides further pasturage.

Hogs and sheep were ineffective in controlling bindweed in Nebraska (204), while in Utah about 90% control of bindweed was secured by hogs in pasture (361). In Iowa trials neither leafy spurge nor field bindweed was controlled with sheep or hogs (19). Workers at the North Dakota Agricultural Experiment Station were successful in cleaning areas of leafy spurge with sheep (175). Likewise, the Minnesota Agricultural Experiment Station has found that sheep may be used to good advantage in destroying leafy spurge. Muenscher (255) also advises use of sheep in controlling leafy spurge under New York conditions.

Chemicals

In 1896 a French grape grower by the name of Bonnet, while applying Bordeaux mixture to his vines, noticed that the leaves on mustard plants in his vineyard turned black when the spray solution fell upon them (41). The news of this effect spread, and soon experiments in weed control with chemicals were numerous. Another early French worker reported the use of iron sulphate and copper sulphate spray to control dodder plants in alfalfa (179), and in 1896 Bolley experimented with copper sulphate as a weed eradicant and was one of the early workers to give methods of using spray equipment (39).

Some spray materials are effective by destroying plant tissue upon coming in contact with it. In general, these sprays are used on annual plants which cannot recover after destruction of their vegetative parts. Many of the sprays which kill by contact are applied in very dilute solutions and do not render the soil sterile. In some cases they are selective in that certain plants are destroyed while others receive little or no injury.

Sulphuric Acid and the Sulphates. For many years sulphuric acid has been used as a selective spray in control of such annual weeds as mustard. In 1911 6% to 10% solutions of it at the rate of 107 gallons per acre were used in grain fields (297), and later work by the same investigator gave other results (298, 299, 300, 301, 302). Korsmo (212) found that a 3.5% to 4% solution would destroy those annual weeds to which the acid would adhere, and he secured increased yields of from 561 to 603 pounds of mixed grains per acre. Other work at the same station also gave good results and showed that atmospheric conditions had little influence upon the effectiveness of the acid when used to destroy mustard in small grains. An hour usually was sufficient for permanent destruction except during cold, wet weather (14).

In California, workers recommend application of a 10% concentration by weight of 66° Baumé commercial sulphuric acid applied at the rate of 130 gallons per acre (129). At an application cost of $3.00 per acre, barley fields infested with mustard showed an increased yield of 50%. Sulphuric acid treatment has reduced lodging of barley (24), and successful use of it has been reported in Arizona (57). Thornton and Durrell (374) state that a 5% solution will kill the majority of weeds encountered on the farm, including dodder, and they believe that the effectiveness of the acid is favored by low humidity and high temperature. From 50 to 200 gallons were required per acre when the weed growth was from four to six inches high.

Blackman and Templeman (37), who secured an average of 90% control of *Brassica arvensis* (L.) Ktze., using a 9.2% concentration of sulphuric acid, noted some cases of damage to the crop. An anonymous worker (7) recommends control of charlock in cereals with a 100 to 150 gallon per acre application of a 7% to 10% solution.

In western Canada, sulphuric acid and copper sulphate have been recommended for economical control of wild mustard in wheat (140, 141). Stinkweed or French weed was controlled by sulphuric acid only. Applications of 30 pounds of copper sulphate (3% sol.) and 60 pounds of sulphuric acid (6% sol.) per acre gave a reduction of 87% to 90% in amount of weed seeds in the crop, and the yields of harvested grain in the treated plots were, respectively, 5.3 and 2.7 bushels per acre higher than in the untreated weed plots. Sulphuric acid controlled common wild mustard, Indian mustard, wild radish, stinkweed, false flax, tumbling mustard and wild buckwheat growing in wheat. Under the conditions of the experiment, Canada thistle, hare's ear mustard, ball mustard, cow cockle, lambsquarters, Russian pigweed and grasses were not controlled. In resistance to sulphuric acid the cereals ranked as follows: resistant—wheat, oats; intermediate—barley, spring rye; susceptible—corn.

Vigor used iron sulphate to control mustard in grain fields (385), and St. John's wort was destroyed with a 5% solution of sulphuric acid (308). Sulphuric acid methods in France are of interest (342). Sourdille (352), for instance, controlled weeds in grain fields with it and ammonium sulphate, and similar results are reported for Italian trials (249).

Suitable equipment for applying sulphuric acid is essential (24, 130, 131). Because of the corrosive nature of the acid it is necessary to use certain resistant materials such as brass, bronze of low zinc content, rubber and special grades of steel or iron made from an alloy of copper, silicon and manganese. It is interesting that while concentrated sulphuric acid may be handled in steel or wrought iron, the diluted acid will corrode both.

Sodium dinitro-orthocresylate. Recently there has been introduced a new type of selective herbicide, an organic dye, sodium dinitro-orthocresylate, sold under the trade name of Sinox. Most experiments have indicated its usefulness in the control of such broad-leaved weeds as mustards. Since the grasses and flax are tolerant of Sinox it may effectively destroy the undesirable mustards and leave the crop uninjured. Sinox has the added advantage of being non-corrosive and non-poisonous in the quantities used. To secure the most effective action, the spray solution should receive an acid salt, such as ammonium sulphate or ammonium bisulphate, to increase the hydrogen-ion concentration of the spray and, in turn, to increase the concentration of the dinitro-orthocresylic acid in the solution (139). Westgate and Raynor (396) rate activated Sinox as equal in effectiveness to any other selective herbicide. They found it to be effective on vetch, corn cockle, sunflower, black nightshade, mustard and wild radish, all weeds that infest grain fields in California. There appears to be considerable advantage in yield and quality of flax produced in plots sprayed with Sinox as compared with untreated areas (176). Favorable results with Sinox were secured also in Oregon where it is recommended as a selective weed killer in wheat, barley, flax, peas and certain grass seed crops, and proves especially effective in the control of mustard growing in flax (164). Similar results have been secured in Wisconsin and Minnesota (332, 402).

Arsenicals. Arsenical compounds have been effectively employed by numerous investigators. In 1892 sodium arsenite was used in Africa to kill prickly pears (242), and both annual and perennial weeds of Hawaiian rubber plantations have since been controlled by it (244). The latter investigator considered the possibility of damage to the soil as a result of arsenic accumulation from the treatments on undisturbed land that had received three sprayings of five pounds of arsenic per acre per year for five years. Under the very heavy rainfall of the area (200 inches per year) the first four inches of the soil contained 0.009% of arsenic and no arsenic was found below this level.

In Minnesota sodium arsenite applied to quack grass at the rate of four pounds dissolved in ten gallons of water per square rod gave a 90% kill. Heavier applications effectively destroyed long-lived perennials but made the soil sterile for several years. The results of these investigations are unpublished.

Crafts and his co-workers in California have done much work on the use of chemicals in weed control. One must remember that soil and climatic conditions in one part of the country may differ widely from those in another section. In California, for example, sodium arsenite has been successful and is recommended for use under certain conditions. Extensive studies have been made in California using a combination of arsenic trioxide and sulphuric acid, the sulphuric acid proving useful as a penetrating agent (82, 83, 85, 87, 89). Four parts by weight of As_2O_3 and ~~one part by~~ weight of NaOH were mixed dry, and three parts by weight of water added. The mixture was then thoroughly stirred until the salts were dissolved, and the thoroughly mixed combination next received five parts by weight of concentrated sulphuric acid. The resulting mixture, diluted with 100 parts by weight of water when used, was sprayed under 100 pounds pressure at the rate of 500 gallons per acre. This treatment proved effective in California but is ineffective in more humid areas. The spray exerts no permanent injurious effect upon the soil. Later studies with a group of 80 California soils corroborate the earlier results of arsenical effectiveness (95). In the fog belt of California four pounds of arsenic trioxide were dissolved in 100 gallons of water, and applications of 300 gallons of spray per acre in October successfully eradicated field bindweed (150).

In Australia one application of 120 gallons per acre of a 6% solution of arsenic pentoxide gave up to 95% eradication of *Lepidium Draba* L. (251, 252, 253). Most effective results were secured when treatment was made after a period of dry hot weather. Some investigators believe pentoxide to be the most satisfactory form of arsenic for herbicidal purposes (66, 344).

Crafts (85), working with four different California soils in the greenhouse, found trivalent arsenic the most feasible chemical for soil sterilization, based on cost. Further greenhouse investigations showed a mixture of white arsenic and sodium chlorate to be useful in soil sterilization (94). In dry areas considerable use is made of the arsenicals to sterilize soils around fence lines, irrigation ditches, *etc.* (97). Because of their very poisonous characteristics, extreme care in use is important (89).

Other investigations of arsenical herbicides include those of McDonald (242) and Morgan (250), and a study which gives evidence that the toxicity of arsenicals is inversely related to their fixation by the soil (320). Little or no effect on soil productivity of several Cotton Belt soils was noted if applications of calcium arsenate were limited to less than 400 pounds per acre (114).

Chlorates. Of the various chemicals used in weed control, the chlorates, particularly sodium chlorate, have been most widely used. As early as 1901 potassium chlorate was employed to destroy prickly pear. As the results were not especially promising, the work attracted little attention. In 1923, dilute sodium chlorate was reported to be effective in weed control (236). A little later, Korsmo destroyed 11 species of perennial weeds by one application of 3.16 gallons per square yard of a 5% solution (213).

Soon after the earliest chlorate investigations, published reports by American investigators appeared. Intensive Kansas studies in the control of field bindweed showed that a 12.5% solution of sodium chlorate was most effective and that treatments should be made about the time the plants were in full bloom (223, 224). In Minnesota, sodium chlorate proved to be the best of several chemicals tested. The proprietary compound Atlacide was more expensive than the straight sodium chlorate and did not give equal results, pound for pound.

While much of the chemical weed control work has been on field bindweed, sodium chlorate has been effective on other weeds. Harper (163) effectively treated Johnson grass with a 12½% solution applied 100 gallons per acre in May and again about August 1. In New South Wales Johnson grass was controlled with a 450 gallons per acre application of a 10% solution (54). One investigator found sodium chlorate and Atlacide to be equally effective in the control of Johnson grass (198). Many other investigations have been made of sodium chlorate as a weed eradicant (12, 68, 84, 85, 90, 105, 154, 171, 183, 186, 188, 227, 232, 235, 245, 257, 271, 279, 310, 322 and 379).

Most investigators favor sodium chlorate over the other chlorates. Magnesium and calcium chlorates have been suggested as substitutes (225). Aslander (15) secured good results on Canada thistle with dry potassium chlorate. A French investigator (133) secured equally good results with potassium and sodium chlorates. Today nearly all workers are using sodium chlorate in preference to the other chlorates.

Numerous problems confront the user of chemicals for weed control. The method, time and rate of application must be determined for each chemical used. Wide differences in effectiveness may result from a variation in the methods of use. For example, in North Dakota dry applications of sodium chlorate were effective in controlling bindweed, provided there was sufficient rainfall to carry the chemical into the soil and the temperature was relatively low to prevent chlorate decomposition (173). Dry applications were effective in the control of Canada thistle in New York (15). Muenscher (259) recommends the dry treatment for perennial weeds in general. Minnesota trials show the dry salt to be as effective as the spray method (402). Use of the dry salt obviates the purchase of expensive spray equipment and reduces the fire hazard greatly, as chlorate is highly explosive when mixed with dust or organic matter, and several disastrous fires have resulted from careless use. Release of the oxygen contained in the chlorate molecule results in a very high temperature and a fire which can not be extinguished with water.

At Cornell fall applications of chlorate are recommended (15). Similar recommendations have been made by other investigators (259, 329, 402). Harper (163) reported fall applications to be ineffective in the control of Johnson grass. In Minnesota applications before August 1 did not penetrate the soil more than two feet, but in treatments after August 1, penetration was greater, being directly correlated with the accumulation of soil moisture (55).

Recommended rates of applying sodium chlorate are not the same in all areas. Aslander (15) destroyed Canada thistles with 200 kgm. per hectare. In Ohio (398) a total of four pounds per square rod was most satisfactory, while in Minnesota (402) the general recommendation is about 500 pounds per acre. If additional treatments are necessary, chemicals may be applied to small areas the following year.

It is generally believed that chlorate enters the plant through the root and thus is most effective when it reaches the soil. Removal of plant top growth may expedite the effectiveness of the chemical (234). The organic matter present may affect the efficiency of the treatment, as chlorate toxicity has been shown to be inversely correlated with soil fertility (91). It was believed that seasonal variations in the response of plants to treatment may be explained on the basis of nitrate concentrations as affected by leaching, temperature, absorption by plants, and nitrification. More recent work at Wisconsin has given similar results (330, 331). Increments of organic matter markedly decreased chlorate toxicity. Soils containing nitrates may be leached to lower levels and thus alter the toxicity of chlorate at different root levels (321). It is possible that the ineffectiveness of chlorate under conditions of high levels of fertility may be due to microbial activity rather than to the presence of organic matter. High organic matter with moisture and warmth results in greater microbial activity and reduced effectiveness of chlorate (264).

Latshaw and Zahnley (223) found that sodium chlorate reduced the nitrifying power of soils, the effect being greater on the later treated soils. Aslander (16) reported no effect upon the ammonification process of soils which had received from 500 to 2,000 p.p.m. of chlorate. Accumulation of nitrates was diminished when the chemical was applied in the fall. There was no influence on the ammonification and nitrification processes the following spring. Chlorate had little or no effect on earth worms and none on protozoa. Species of *Penicillium, Aspergillus* and *Fusarium* grew on top of hay infusions containing N/10 $NaClO_3$, and numerous bacteria developed in the solution. Another investigator (217) reports that chlorate was injurious to microbiological life in the soil and to nitrification. It was shown that both inoculation and treatment with ammonium sulphate gave increases in survival of red clover plants on land previously treated with chlorate (152). Decomposition of chemicals in the soil may exert a marked effect on soil microbiological activity and subsequent growth (42).

Hurd-Karrer (192) has reviewed the literature on soil conditions affecting the herbicidal action of chlorates. In Germany no unfavorable effects of sodium chlorate upon soil reaction or physical structure were evident (292). Toxic effects on soil under Canadian conditions are discussed by Bowser and Newton (42).

Weeds growing under irrigated conditions present different problems than those in more humid areas and require a different amount of chlorate for effective control (185). Chlorate toxicity is usually greatest in acid soils and least in alkaline soils. Soils which were limed (pH 7.5–8) showed the least toxicity (191).

The tolerance of susceptible plants to chlorate is a measure of the quantity present in the soil. *Tradescantia virginica* Wats., hemp, tomatoes, beans and sunflowers were good testers, since all were more or less injured by less than 10 p.p.m. in the soil (356). The resistance of crop plants is of great importance, since under farming conditions the aim is to return the treated land to productivity as soon as possible. Hurd-Karrer found barley, sunflowers and spinach to be most sensitive to chlorate in greenhouse tests. Wheat was nearly as tolerant as oats; rye was definitely less tolerant and barley the most susceptible of the small grain crops. Flax was nearly as tolerant as oats. Vetch, sweet clover, and alfalfa were among the more resistant crops, while the sorghums tested were susceptible (190).

No discussion of chlorates would be complete without a consideration of the poisonous characteristics of the salt. When fed in measured doses, from 18 to 400 grams were injurious to average sized dairy cows (123). A full grown steer became ill after having access for two weeks to a lick of one part bonemeal and two parts sodium chlorate (333). Care must be exercised when animals graze on areas treated with herbicides (254).

Other Chemicals. Many types of chemicals have been tested by research workers in their efforts to find an effective but economical weed eradicant that is free from the many disadvantages of those herbicides generally used.

Carbon bisulphide, a volatile liquid whose gas spreads through the soil, has been used as a weed killer for several years. In Colorado it proved successful in destroying several long-lived perennials (317). In general, results have been good in dry areas but not so encouraging in more humid regions. A prime disadvantage of carbon bisulphide is its explosive nature which renders its use extremely dangerous.

Harvey (166) is generally credited with doing the first experimental work with ammonium thiocyanate as a weed herbicide. Under Minnesota conditions applications of 10 pounds per square rod made the soil sterile for at least four months. A two pound application sterilized the soil for two to four weeks, and subsequently gave a definite stimulation of growth, due to the release of nitrogen. Applications of 10 pounds of the salt dissolved in water to each 1,000 square feet usually killed all annual weeds (98). Several investigators have tested the thiocyanates and found them generally successful with shallow rooted weeds but ineffective in destroying deep rooted persistent perennials. Further studies are needed to determine their place as possible adjuncts to other herbicides. Studies have shown ammonium thiocyanate to be inferior to chlorate in destroying leafy spurge (26). The chemical exerts definite effects upon nitrification and soil organisms in general (327), and it has been reported as toxic to livestock, especially cattle (267).

Boron has been suggested as an economical herbicide, and many recent investigations indicate that it has a place in weed control. California investigators state, on the contrary, that boron compounds will find but limited use in weed control (93). Under their conditions they believe that a combination of sodium chlorate with some one of the cheap boron ores may prove desirable. Trials in North Dakota showed no advantage of borax over other herbicides (174). Unpublished results at the Minnesota Station indicate that borax may have a place in weed control, as generally good results have been secured in the eradication of long-lived perennials. An unsolved problem is the length of time the boron may sterilize the soil and prevent the growth of desirable plants. Its non-poisonous and non-inflammable characteristics make its use free from the hazards of chlorates.

Blackman (36) reported that aluminum salts, copper salts, sodium bisulphite and ammonium thiocyanate were ineffective. Both sodium bichromate and sodium arsenite were less effective than sodium chlorate. Similar work at Cornell (15) showed sodium thiocyanate, sodium cyanide and sodium arsenite to be inferior to chlorate. One investigator proposes the substitution of sodium bromate, in part, in a mixture of sodium chlorate (180). More than 150 chemicals were tested for the eradication of many species of *Ribes* (273). Paint was reported as effective in killing weeds (309). Certain chemicals have been used as catalysts to increase the effectiveness of the principal herbicide. Salts of magnesium, cobalt, nickel and vanadium pentoxide were used to intensify the action of sodium chlorate (101). Bates (31) also secured improved results from the use of vanadium pentoxide as a catalyst.

Some investigators have used fertilizers to destroy weeds (231, 338, 339). Kainite destroyed poison ivy when applied to the wet foliage (63). Sodium dichromate lowered the yields of wheat in the season applied but tended to increase yields the following year (266). Sodium ethyl xanthate and ethylene oxide have been proposed as weed eradicants (167, 169). Tetrachlorethane was effective in destroying field bindweed (22). Water hyacinth was checked by sodium pentachlorphenate (182). Quack grass and field bindweed were not destroyed by petroleum oils containing differing amounts of furfural (58).

Zinc sulphate was effective in destroying germinating weed seed roots without injuring the evergreen roots with which they were growing (6). Thallium sulphate proved very toxic in sterilizing soils (88). Liquid chloropicrin has been used to destroy weed seeds in composted soil (184) and weeds in turf (144, 248). This chemical exerted a marked effect upon nitrification and ammonification in the soil (357). Other trials with miscellaneous herbicides show considerable difference in toxicity (35, 47, 72, 73, 74, 75, 76, 265).

How Chemicals Kill

According to Aslander (16), dilute sulphuric acid sprays do not plasmolyze plant cells. The acid penetrates into the cells, unites with the magnesium atom in the chlorophyll molecule and decomposes the protoplasm. The chloroplasts are destroyed but the cell walls usually are not affected.

Arsenical sprays kill the plant parts with which they come in contact, and their action in killing the roots of perennial weeds has been explained as being due to transport of the poison through the stems into the roots. Within the fog belt in California, arsenical sprays applied to bindweed in bloom killed many of the plants (150). After a period of dry hot weather, stems of bindweed cut under eosin solution carried the fluid to a considerable depth (92), and it was believed that the movement indicated subatmospheric pressure. "The poisonous nature of arsenic necessitates its transport through non-living channels in lethal concentrations. This practically limits its movement within the plant to the xylem and prescribes a high water deficit and particularly favorable conditions for application of the spray. These conditions are seldom met in actual practice".

Killing by leaf and stem absorption has been classified as local or remote, and it was believed possible that the arsenic could be carried downward into the roots along with elaborated plant food in that direction (196). In this way better killing at about the time of bloom than at other times was explained. Many factors influence the use of chemical weed killers (243).

The roots of plants to which chlorate had been applied were practically free from starch, while untreated plants had an abundance of reserve material in their roots (223). It was believed that the chlorate interfered with the photosynthetic activity of the plants and compelled them to draw on the reserve food supplies.

Harvey (165) offered the following explanation as to how ethylene oxide and sodium chlorate kill plants: "The tissues turn black and the cells die. Evidently the respiratory chromogens are so completely oxidized that they cannot function. They are oxidized to the pigment forms peculiar to each tissue".

The catalase activity of field bindweed roots growing in sodium chlorate treated soil was found to be lower than that in the roots of untreated plants. "It is probable also that the chlorate ion inhibits the other enzymatic activities of a plant in a similar way. It is possible that the more slowly acting but higher toxicity of the chlorates is due to the gradual decomposition within the plant tissues accompanied by a liberation of nascent oxygen" (263).

In *Nitella* there was no accumulation of the chlorate ion within the cytoplast when injury to the cells took place, and the quantity of the chlorate or chlorate ion fixed by the protoplast was very small. This indicated that the concentration of the chlorate was not materially lowered in the course of toxic action, a condition that would allow the chlorate solution to penetrate relatively easily and probably reach the vascular tissues of the plant. "Apparently the typical chlorate action may be exerted over a wide range of the concentrations and, after the initial plasmolytic action, the concentration does not materially affect the rate of injury". Addition of ammonium chloride to sodium chlorate intensified somewhat the chlorate action; on the other hand, addition of calcium chloride retarded the action (272).

Loomis *et al.* (234) also discuss absorption and movement of sodium chlorate. The action and problems relative to its use as an herbicide are explained by Crafts (86). The effectiveness of spray applications in control of field bindweed is dependent upon atmospheric conditions (200), and the poisonous ingredient is not the chlorate but the hypochlorite ion (253). Using copper nitrate solutions, Silversides reports on the method of penetration of herbicides into plants (336).

WEEDS OF THE TURF

Probably no other group of people is more aware of the weed menace than those who care for lawns. In general, the species which must be fought by the lawn caretaker are few, but some of them are so persistent that they prove real menaces. In addition to the following more specific treatments of the subject there are several general contributions (62, 103, 168, 247, 380, 393, 395, 404).

In a recent complete discussion of weed control in lawns, the primary requisites are clean cultivation before seeding, use of pure seed and clean top dressing, and adequate fertilization (261). The authors recommend sodium chlorate, one and one-half to two ounces in one gallon of water per 100 square feet, applied in early spring or late fall when the weather is cool. When crabgrass is in the seedling stage, one pound of sodium chlorate should be mixed with 20 quarts of sand and spread over 1,000 square feet. Seventy-five to 100 pounds of dry granulated iron sulphate in 50 gallons of water per acre applied as a fine spray may be used as an alternate in four to six applications at intervals of about 10 days.

Other workers, too, have reported on the use of sodium chlorate in controlling lawn weeds (148, 208, 262, 378). Fall applications are recommended for renovation work at the rate of one to one and one-half pounds per 1,000 square feet (391), and applications of two pounds per 1,000 square feet with three successive applications have proved effective on crabgrass and other common lawn weeds.

Applications of 1,000–2,000 lbs. of calcium cyanamide per acre on Bermuda grass lawns from December 1 to March 1 were successful in destroying annual weeds, but the chemical should not be used on bluegrass lawns (368, 369). Another worker recommends calcium cyanamide for the control of dandelions (341), while others report that lead arsenate was effective in controlling chickweed and crabgrass (270).

A 2% solution of sodium fluoride plus 1% soap destroyed or injured crabgrass with little injury to bluegrass (240). Another worker secured effective control of dandelion with copper nitrate (336), and copper sulphate, one pound per 100 square feet, was effective in killing certain weeds (181).

Ammonium sulphate, a commonly used nitrogenous fertilizer for lawns, is widely employed also in weed control. Rosette types were controlled by it (314), and addition of ferrous sulphate eradicated mosses and clovers. Six pounds of $(NH_4)_2SO_4$ per 1,000 square feet may be applied in the early morning when the grass is wet with dew. Application in early spring or late fall, with water applied after a day or two, is a recommended practice (261). One investigator used it successfully at the rate of one-half ounce per square yard, either dry or as a 5% solution (one-half pound per gallon of water), to destroy weeds and white clover in lawns (69), and others have secured similar results (104, 270, 390). Addition of a soft soap as a spreader has been found to increase the effectiveness (120). Lead arsenate applications in October, December, February and April were effective on crabgrass, 20 to 25 pounds per 1,000 square feet usually giving almost complete control (394). Any potential bad after effect was overcome by liberal use of nitrogenous fertilizers.

One-half gallon of water-white kerosene (color Saybolt 23) to each 100 square feet of lawn has helped to control crabgrass, mouse ear chickweed and dandelions (402). Loomis (233) advises the use of straight-run kerosene with a boiling point range of 180° to 250° C. and an unsaturated hydrocarbon content of not more than 4% as a means of destroying dandelions in bluegrass lawns. The kerosene is applied at a rate of 200 gallons per acre two months before the end of the growing season, since it requires from two to three weeks for the grass to recover and this allows about six weeks for it to build up reserves for the winter. Dandelions were also controlled by using 126 cc. of mercurated ethyl stearate in 6½ gallons of kerosene applied to an area of 1,000 square feet (153), while other investigators used dichloroethyl ether (372) and paraffin oil (268). Others have secured good results in the control of dandelions and broad-leaved plantain by using Meo–181 and high grade kerosene, the best effects with least damage to bluegrass being secured when treatment was made between September 20 and October 1 (230).

Lawn Sinox was best for the control of chickweed when applied as a spray at a rate of four and one-tenth gallons per square rod in a 1:64 dilution.

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FUNGUS AND BACTERIAL DISEASES OF INSECTS AS FACTORS IN BIOLOGICAL CONTROL

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INTRODUCTION

In comparison with investigations of entomophagous parasites (insects parasitic on other insects), experimental work on entomogenous parasites (fungi or bacteria parasitic on insects) in relation to biological control has received relatively little attention. Consideration of the latter subject, except for a few microorganisms, has not gone much beyond the observational and descriptive stage, and only a small number of well-controlled experiments have been carried out. The reason for this appears to be partly that in the past period of extreme specialization this subject has become a kind of "no man's land" between the entomologist and the plant pathologist or applied mycologist.

One apparent cause for neglect of the field has been the hasty generalization that because of failures to get outstanding results with certain fungi in initial trials, the whole field has little promise of practical results. Another is probably the preoccupation of both entomologist and plant pathologist with problems which they considered more closely related to their special fields. Mycologists, however, have in the meantime done considerable descriptive work on the occurrence and morphology of these entomogenous fungi.

An attempt will be made here not to give a complete review of the literature but to discuss some of the most important contributions and to use representative examples, mostly from entomogenous fungi and bacteria on the citrus insects, these being best known to the author.

The literature on these organisms is widely scattered. For part of them, Petch has published a large number of papers, especially on taxonomic relationships of entomogenous fungi (40–57), and many other sources are extant (2, 4–6, 19, 22, 36, 39, 68, 71). There are also comprehensive lists of fungi on insects (14, 34), and numerous references to bacteria in and on these hosts (38, 73, 74).

GENERAL CONSIDERATIONS

In biological control of insects two opposing forces of survival, as pointed out by Chapman (11, 12), come into play, namely, "biotic potential" or inherent ability of the insect to reproduce and survive, and "environmental resistance", constituting the physical and biological factors that oppose survival. For insect survival the parasitic fungi and bacteria are a part of this environmental resistance and may constitute a small or a large factor in opposing survival, depending on the degree of parasitism and the physical conditions influencing distribution and infection. The opposing forces of biotic potential and environmental resistance also operate on the fungi themselves, and the insect's resistance to infection may be a part of the environmental resistance for the fungus. Some of the factors of biological control, especially as related to entomophagous parasites of insects, have been discussed by others (62–64, 76).

Gradation of Parasitism. Entomogenous fungi are variable in their nature and relationship to their insect hosts, as are the plant pathogenic fungi to their plant hosts. In parasitism of insects as in parasitism of plants there is apparently a gradation from the strongest and most effective parasites down to those which are very weak and indirect. Among the weak and indirect parasites, occur species about which it is difficult to tell whether they are slightly parasitic or merely living upon the insects after their death. Many that are distinctly parasitic at one stage are capable of completing their reproductive stage while growing upon the insect's body after its death. It is therefore unsafe to draw conclusions about the efficiency of the entomogenous fungi as a whole from a few examples such as the chinch bug fungus, most often referred to in publications on biological control. Four categories of parasitism may be mentioned with examples:

(*a*) Some entomogenous genera, *e.g.*, *Sorosporella* and *Empusa,* are obligate parasites and propagate only on or within the body of the live insect. As soon as the insect dies they usually form resistant resting bodies or spores which propagate again only when they come in contact with another live insect.

(*b*) Other entomogenous genera, *e.g.*, *Aschersonia, Aegerita* and *Nectria,* are parasitic or semiparasitic whenever the spore or fungus mycelium comes in contact with the insect, and under suitable conditions may kill the insect. They may also live a saprophytic existence on the bodies of the insects after their death. Some of this second kind may kill the insect more rapidly and be more effective in epidemics than some of the obligate parasites.

(*c*) Still other entomogenous fungi may be weak parasites, dependent on a special stage or weakened condition of the insect for their infection and propagation.

(*d*) Some species of at least one genus, *Septobasidium,* appear to have a partial symbiotic relationship to certain scale insects (15). The colony as a whole appears to be protected by a covering of mycelium, although at times part of the larvae appear to be parasitized by the fungus.

A fifth category cannot be considered as parasitic at all; the members live as saprophytes on the bodies only after the insects have died. Most species of *Cladosporium* found on insects are thought to be in this class, although some may possibly be semiparasitic under certain conditions.

Conditions Influencing Effectiveness. Although a number of these parasitic entomogenous fungi under favorable conditions develop conspicuous pustules or sporulating structures after they have killed the insect by invasion of its body, this development of conspicuous evidence of their presence may be arrested by drying conditions after infection, so that the insect body is desiccated or rendered unfit to support this later development of the fungus. Thus, what is often referred to by observers without careful microscopic examination as "natural mortality" or "unexplained mortality" may be due to arrested stages of fungus attack without visible evidence, even with a hand lens. Other cases of "unexplained mortality" may be due to parasites which live internally and rarely if ever show on the exterior.

In the effectiveness of these entomogenous fungi under natural conditions there appear to be a number of contributing factors:

(*a*) In general, the fungi are favored by rainy, wet weather or high humidity, or by conditions on the plant and insects where moisture is sufficient for spore germination.

(*b*) Most entomogenous fungi appear to be favored by moderate to warm temperatures, although the best temperatures for propagation vary greatly between species.

(*c*) Degree of light may be another factor; most fungi apparently are favored by shade, but others apparently depend on light for spore production, as the brown fungus, *Aegerita webberi* Faw. (20).

(*d*) Entomogenous fungi are dependent on favorable conditions for their distribution at the time when the insects begin to increase toward an injurious density. Some are spread by winds with or without driving rains, such as *Beauveria, Aspergillus, Metarrhizium* and *Verticillium,* while others are apparently dependent on contact with migrating insects of the same or different species, such as *Empusa fresenii* Now. and *Sorosporella uvella* (Krass.) Gd.

(*e*) Dense insect populations favor epidemics of some fungus species; a few, however, for example, *Aegerita webberi* Faw., the peculiar brown fungus on citrus, are only partly favored by density of the citrus whitefly larvae; this fungus has, in addition to internal hyphae, external mycelium that spreads over the surface of the leaf and may in suitable weather extend long distances on the leaf from one larva to another.

(*f*) Some organisms appear to be able to parasitize the insect more readily under one condition of nutrition of the host plant than under another condition (66, 67, 79).

It follows from these considerations that some of the entomogenous fungi are to be regarded, under certain conditions, as important factors in the biological control of insect pests, while others are apparently inefficient or of minor importance. Some of the same factors appear to govern their effectiveness, as those which influence the effectiveness of different entomophagous insects (63, 64, 76).

Artificial Use in Control. Whether the efficiency of parasitism can be increased by artifical means in any particular case depends, as it does in insect parasites, on some of the following considerations:

(*a*) If the conditions for natural distribution of a fungus are such that there is the maximum number of spores capable of infecting the maximum possible number of insects under prevailing environmental conditions, then no added results could be expected from artificial distribution without at the same time changing the conditions. A relationship of this kind may be known as the "saturation point" for insect and fungus. This saturation point was probably the relationship of *Beauveria globulifera* (Speg.) Pic. to the chinch bug during the experiments where no increased mortality resulted from the distribution of additional spores (7). A saturation point might be expected most of the time from the abundance of wind-borne spores which such a fungus is capable of producing.

(*b*) When conditions are such that natural distribution is inefficient, *i.e.,* when this distribution is retarded or lags behind that necessary for maximum infection under prevailing conditions, then artificial distribution would be expected to increase the degree of infection. Whether artificial distribution would constitute an economical measure of control would depend, as it does with entomophagous insects, on what degree of increase could be effected by this means and its cost. It is believed that the natural distribution of a number of these fungi often lags far behind the possible maximum degree of infection, as in the aphis fungus of Florida, *Empusa fresenii* Now., which appears to depend for propagation on spores that are not readily distributed by wind and on the time of the insect's migration (23, 81). Artificial distribution preceding migration would appear to be suggested.

In certain situations in Florida, after a dry period or season the previous year, *Aschersonia* and *Aegerita* are not sufficiently abundant to efficiently infect whitefly larvae without artificial distribution at the beginning of the rainy periods in summer, usually June or July (87). If the "saturation point" has not been reached, then artificial distribution would increase mortality.

(*c*) As with insect parasites on insects, so with the fungus parasites, where the saturation point has been reached for natural infection under a given set of conditions, there is still some possibility of artificially manipulating or changing the natural conditions to shift the saturation point and to increase infection. Changes may be made in humidity by overhead irrigation to increase the moisture on the surface of leaves or fruits for a short time, or by other devices, as the growing of intercrops. This latter has been suggested in Florida in respect to the aphis fungus, *Empusa fresenii* Now. (81). With fungus diseases on plants it is known that such changes in conditions (rapid weather changes, *etc.*) do shift the saturation point and greatly increase infection (25).

(*d*) There is also the possible increase or decrease of susceptibility of an insect to infection because of changed nutritional conditions influenced by the nutrition of the host plant. A case of this kind is mentioned later under the bacterium of the citrus red scale (66, 67).

One feature of this saturation point for infection is that it can not be certainly known that it is reached by finding the organism generally distributed in a given locality. This point may be reached at one particular time and not at another. Moreover, in many organisms, fungi or bacteria of common plant and animal diseases, it has been shown that the amount of infection may be greatly increased by the nature of the inoculum. Density and size of the inoculum of bacteria, or the "spore load" of fungi, is of great importance in breaking down resistance to infection (25).

While the use of these organisms in pest control has so far been limited, the future possibilities in their use, as of insect parasites on insects, depend, it would seem, on (*a*) devising efficient, economical methods of spread when there is an unsaturated condition of infection; (*b*) devising economical means of changing the environment on or about the plant to increase infection, *i.e.* to artificially shift the saturation point upward in order that the organisms present or being applied may have better conditions for infection; (*c*) changing the nutrition of the plants and concurrently that of the insects to make them more susceptible to infection by the parasitic organisms present or being applied.

FUNGUS PARASITES OF INSECTS

Chinch Bug Fungus. This fungus, *Beauveria globulifera* (Speg.) Pic. and formerly known as *Sporotrichum globuliferum,* is a conspicuous species forming a white growth over the dead bodies of insects. It has perhaps been referred to most often as a typical example of an entomogenous fungus, having been reported as occurring on at least 60 species of insects of various families in North America. Its parasitism, such as it is, is apparently not specialized, as is that of many entomogenous fungi. It is an example of fungi having wind-borne spores produced in great profusion, and is capable, therefore, of wide distribution in a short time, readily producing a saturation point for infection which is not so easily attained by certain other entomogenous fungi of more highly specialized parasitism. This probably accounts for the reported failure to produce increased mortality by its artificial spread to the chinch bug, *Blissus leucopterus* (Say) (7). Petch, as the result of culture work on species of *Beauveria,* found numerous strains and concludes that "very many of the records of the host insects of the various species of *Beauveria* are open to question" (50). It would seem unsafe, therefore, to make generalizations from this example alone as to the value of entomogenous fungi.

Lefebvre, working with two species of *Beauveria* on the corn borer, found that *B. Bassiana* (Bals.) Vuill. was much more virulent than *B. globulifera* (Speg.) Pic. These two had been confused as one species by some authors. He concluded that the former had favorable potentialities for at least partial control of corn borer larvae, since they are exposed most of their lives to a relatively moist condition within succulent tissues (33).

Green Muscardine Fungus. Another conspicuous fungus with numerous, powdery, wind-borne spores is the green muscardine fungus, *Metarrhizium anisopliae* (Metsch.) Sor. This fungus, which is related to the penicilliums, has been identified on more than 60 species of insects, mostly beetles, in North America. It has been grown in pure cultures, and the spores in large quantities applied with a dusting machine in order to start epidemics earlier than would otherwise occur. Rorer in Trinidad found distribution of this fungus early in the season of value in increasing the mortality of the sugarcane froghopper, *Tomaspis varia* (F.) (61). For the May beetles, *Phyllophaga* spp., in Porto Rico, however, Stevenson concluded that its artificial distribution did not increase insect mortality sufficiently to make this practice of practical value (75).

Sorosporella of Cutworms. In contrast to the chinch bug fungus, the cutworm fungus, *Sorosporella uvella* (Krass.) Gd., a less known organism, completes its entire development in the body of its host, producing neither external growth nor wind-borne spores, and is an obligate parasite. As Speare (70) points out, while most mycologists are likely to overlook a fungus of this kind through a lack of familiarity with insects and their habits, entomologists, because of their lack of knowledge of mycology, usually give them at best scant attention. The mortality resulting from their attack is usually noted in such general terms as "mortality due to environmental conditions", "natural mortality" or "unexplained mortality". Certain yeast-like bodies known as blastocysts, produced within the blood of the insect host, are carried in the circulation to all parts of the body, making the parasitism complete. Cutworms showing no external sign when dissected, are found to contain a brick-red powdery mass within the larval shell. This mass is made up of spherical reddish-colored resting spores. There is no doubt of the parasitism of the organism whenever the larvae become infected. Inoculation experiments on vigorous larvae show high infectivity of the spores which cause death in less than 10 days (70).

Fungi on Whiteflies of Citrus. Important examples of entomogenous fungi occur on species of whitefly larvae, *Dialeurodes* spp., in Florida. The following are most commonly found and a number of others occasionally: *Aschersonia aleyrodis* Webber, *A. goldiana* (S. & E.), *Aegerita webberi* Faw., *Fusarium aleyrodis* Petch, *Verticillium cinnamomeum* Petch. Some of these have been artificially distributed as means of biological control (1–6, 22, 87). It has been suspected that certain bacteria may also be parasitic on whitefly larvae, but no observation of their occurrence except in a minor way in connection with natural mortality (83) appears to have been reported.

When these organisms are present in certain moist situations in low hammocks of shaded situations in Florida and semitropical countries, the *Dialeurodes* species on citrus are practically controlled during some years. Morrill and Back (36), although concluding that in most orchards in Florida natural control can not be relied on, state: "There are, however, certain circumstances under which fungous parasites may be used to advantage. First may be mentioned the comparatively few citrus groves located in hammocks, with trees growing without regularity and with conditions such that fumigation or spraying with insecticides would be impracticable".

The difference of conclusions from experiments between Morrill and Back (36) who found no conclusive evidence that mortality of whitefly larvae could be markedly increased by artificial distribution by spraying with spores where the fungi were already present, and Berger (2, 4, 5) who found good evidence that it could be increased greatly by spraying spores at the proper season, may possibly be explained on the following assumption. The former probably experimented at a period of approximate saturation point for infection, while the latter probably experimented at periods of unsaturation or of lag in possible infection for prevailing conditions. If this is true it would explain the differences in results.

Use of these fungi to increase natural control has been made principally in Florida (2, 87). The principle methods developed have consisted of simply spraying a suspension of the spores in water into trees infected with the insects. In some cases, fungus-bearing leaves were pinned to fungus-free leaves; in other cases, twigs containing fungus-bearing leaves were tied on branches of fungus-free trees (60). The brown fungus of the whitefly, *Aegerita webberi* Faw., soon after its discovery and before it had spread widely, was sometimes spread by placing young fungus-bearing trees so that their branches would intermingle with fungus-free branches of other trees (88). The source of material for spores to be sprayed was either leaves containing numerous well-developed pustules of the fungi or pure cultures containing numerous spores. The moist season of summer, according to Berger (4), was the most effective period for parasitizing the insects with spore suspensions.

One of the fungi, pure cultures of which are most used for biological control in Florida, is *Aschersonia aleyrodis* Webber. It appears as reddish pustules in and on the larvae. Webber followed with extensive observations the early spread of this fungus in certain orchards which had previously been severely injured by larvae of whiteflies (88). He refers to the striking recovery of trees coincident with the spread of this fungus after its first appearance in 1892. The population of larvae was so reduced in three years that no fruits had to be washed. Later, following the discovery that this fungus could readily be grown in pure cultures on sweet potato slices (18, 19), Berger developed a method of growing large quantities of this fungus and distributing it in pint and quart bottles for use in spraying trees with spore suspensions (2). *Aschersonia goldiana* Sacc. and Ellis is also being grown in the same way.

An important unusual type of entomogenous fungus also used for biological control of whitefly larvae in Florida is Webber's brown fungus, *Aegerita webberi* Faw. This species, in addition to a brown stroma that finally occupies the place of the larvae, has long colorless hyphae that extend over the entire under surfaces of leaves, killing every larva and then extending over the edges onto the upper surfaces and even along stems to the next leaves. Probably a light relationship causes the so-called sporodochia consisting of inflated cells to form only on the upper surface. This cluster of inflated cells which contain appendages becomes detached and is carried by the wind, to function as spores (20).

The first spread of this fungus at Manatee, Florida, was described by Webber in 1897 (88). It was first found in March in a part of a five-acre orchard as a center with no trace in other orchards in the same sections. In nine months, or by December, it had spread with surprising rapidity so that it was difficult to find any living specimen of whitefly larva in the same five acres. By the following year the fungus had spread over a radius of about two miles, reducing the larvae coincident with its spread. Larvae of all stages were infected. Fawcett later proved experimentally the infectivity of the sporodochia, observing effects in nine days (20). The hyphae first appear in the interior of the larvae and the brown stroma burst through the edges in about 16 days. If weather is dry subsequent to infection, the dead larvae with interior hyphae may be desiccated and would then be classed by a casual observer as "unexplained mortality".

No method of successfully growing this fungus in pure cultures in large quantites has yet been developed, but its distribution has been effected in various ways, such as pinning leaves with pustules onto leaves containing live whitefly larvae, grinding up the pustules and stirring in water sprayed on the trees, or planting young fungus-bearing trees in such a way as to have the leaves intermingle. If leaves have the sporodochia abundantly developed on them, stirring leaves in water to get off the sporodochia and spraying the resultant suspension on whitefly-infected trees readily spreads the fungus. As to the effectiveness of this fungus under certain conditions, Morrill and Back (36) state: "The brown fungus has been so effective in controlling whitefly in certain low-lying hammock groves in Lee County [Florida] that it must be conceded this fungus has made artificial remedial measures unnecessary".

Fungi of Scale Insects. Scale insects under certain conditions of temperature, humidity and probably light and other factors may be attacked by a number of different fungi (39, 42. 60, 68). If conditions are unusually favorable in low-lying localities, as in Florida, most of the scale insects appear to remain at a low density level, doing little damage to the trees or fruit. During the dry season, however, in Florida generally during the winter and spring months in higher, drier localities or regions exposed to wind, these insects become very abundant, unless control by natural enemies is supplemented by insecticides.

Evidence has been reported in Florida which shows that not all the increase of scale insects following bordeaux spraying is due, as previously thought (21, 24), to a killing of the fungi. It is found that road dust, lime and other materials not considered as fungicides also cause large increases there in purple scale insects and spider mites.[1] Several authors have concluded from spraying experiments in Florida that these increases can not be entirely attributed to prevention of fungous infection but to the effect of residues (37, 77, 78, 79). The diversity of opinion on this subject has been well stated (27): "Some believe that elimination of the fungi is the major factor; others, that the inert granular residue contained in the spray is the most important factor; and still others, that the increase is the result of the combination of the two factors" (27). The authors referred to present results in spraying trees infested with purple scale in Florida, which lead them to conclude that "the most important factor causing abnormal purple scale, *Lepidosaphes beckii* (Newm.), increase following use of fungicidal sprays is the inert granular residue content of the spray".

In connection with the interpretation of the results of these experiments with residue-producing sprays, certain conditions should be mentioned:

(*a*) The possible effect of the non-fungicidal and fungicidal materials on the tree itself in making conditions more favorable or less favorable for spread and infection of the fungi or for propagation of the insects (79).

(*b*) The possible effect of the material on the insect itself, its nutrition and development in making the insect more resistant or susceptible to infection by the fungi (58, 59).

(*c*) The possible effect of the material on the spread or infection of the fungus, even though the applied material may not kill the fungi already there.

(*d*) The possible effect of weather or other conditions immediately after application of the materials on the fungus and on the insects.

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[1] Certain dusts containing lime also result in increases of California red mite, *Paratetranychus citri* McG., (26), but there appears to be no evidence that purple scale in California is increased by lime or by bordeaux.

It would appear that there is a complex of possible fluctuating factors that need to be unscrambled by experiments with controlled conditions for the insects, for the fungi themselves, and for the complex fungus-insect relationship, before it can be decided what part is played by the deposits or residues from applied materials, by nutrition of the tree and thereby nutrition of the insects, and by the parasitic organisms. Until such experimental work has been thoroughly carried out, the subject must largely remain in an observational and descriptive stage.

Many diverse entomogenous fungi are found on citrus scale insects (22, 42, 87). The most common are species of *Sphaerostilbe, Nectria, Podonectria, Myriangium, Hypocrella, Verticillium* and *Cephalosporium.* One of these, *Podonectria coccicola,* was one of the first entomogenous fungi noted in Florida. Hubbard in 1885 illustrated and described it as the "bark fungus" (28). Watson and Berger believe it to have probably been the main factor that reduced the destructiveness of the Glover scale, *Lepidosaphes gloverii* (Pack.), after this scale had been introduced into Florida at St. Augustine in the thirties of the last century (87). Trees at first were killed back each year by severe infestation, but after some years the scale was reduced by some unknown cause and the trees recovered. The reduction is now believed to have been caused, in part at least, by this fungus.

Well-controlled experimental work has been done on only a few of these scale-inhabiting fungi. Watson dipped branches of small citrus trees heavily infested with non-infected Florida red scale, *Chysomphalus aonidum* (L.) into spore suspensions of *Nectria diploa* (B. and C.) in April at Gainesville, Florida (86). By November every scale was killed. Viegas has reported experiments showing that *Cephalosporium lecanii* Zimm., when sprayed as spore suspensions, is an effective parasite in reducing green scale, *Coccus viridis* (Green), on coffee (82).

Fungi of Mealybugs. Species of *Aspergillus, Entomophthora* and *Empusa* are found as important entomogenous fungi on mealybugs. *Aspergillus parasiticus* Speare has been shown to be highly parasitic on sugar-cane mealybugs in Hawaii, and its effectiveness in killing young, freshly hatched larvae as readily as older ones has been experimentally demonstrated (69). It has been effective also in Puerto Rico (29). In California, pure cultures of an unnamed species of *Aspergillus* produced 100% mortality in healthy mealybugs, *Pseudococcus gahani* Green and *Phenacoccus gossypii* (T. & Ckll.), at 21° C. in 72 hours and at 29° C. in 36 hours, while a large percentage of controls remained alive under the same conditions in a non-parasitic strain of *A. flavus* Link (8). This California mealybug fungus had given trouble in warm non-ventilated insectaries by attacking mealybugs being propagated on potato sprouts for rearing the entomphagous beetle, *Cryptolaemus montrouzieri* Muls., for liberation in citrus orchards (65).

Entomophthora fumosa Speare is considered by Speare as one of the chief factors, especially during periods of summer rains, in the natural control of the citrus mealybug, *Pseudococcus citri* (Risso), in Florida (71).

Fungi on Aphids. On aphids, species of *Empusa* and *Entomophthora* are highly parasitic and common. One of the well-known species is *Empusa muscae* Cohn, the cause of the so-called "frosted flies". Another species, *Entomophthora chromaphidis* Burger and Swain, was reported to parasitize about 88% of the walnut aphis, *Chromaphis juglandicola* Kltb., during one season (9).

The most important one on citrus is *Empusa fresenii* Now. This fungus is reported to be the chief factor in the control of the citrus aphid, *Aphis spiraecola* Patch, in Florida. Insects killed by the fungus are attached to the leaf solely by their proboscis, and appear as if standing on their heads. The abdomen and thorax become coated with tan-colored to light smoky brown, glistening spores. The interior body material is almost replaced by fungus. The spores are not wind-borne but depend for infection on migration of the insects which was found in Florida to be up to 500 yards (23, 30, 31, 80, 81). A colony of aphids is destroyed in a few days after the fungus attack.

Spicaria on Cottony Cushion Scale. Spicaria javanica Bally, forming loose white masses of mycelium covering the bodies of cottony cushion scale, *Icerya purchasi* Mask., is reported in Puerto Rico to have completely killed out this insect in certain orchards well protected by hills and bamboo windbreaks (90). The scales were not seen for at least seven years or during the time of observation, though in other places with less perfect windbreaks, the insect multiplied. The effectiveness of this fungus as a supplement to the entomophagous beetle, *Vedalia,* in Florida when the weather is warm and moist has also been reported (87).

Fungi on Mites. Mites are known to be parasitized by fungi. Charles (13) has described the fungus *Rhinotrichum depauperatum* found on the spider mite, *Paratetranychus yothersi* McG., on *Piaropus crassipes* (Mart.) Britton in Florida. This fungus consists of an effuse, cobwebby, white to pale gray mycelium in which the mites are enmeshed. The mites were killed experimentally in 18 hours when exposed to this fungus. The question arises as to whether the citrus rust mite fungus may be something of this nature.

An undetermined fungus has been reported on the citrus rust mite, *Phyllocoptes oleivorus* Ashm., in Florida. Speare and Yothers state that annually since 1912 rust mites had been observed to disappear as if by magic sometime after the beginning of the rainy season. This is usually the last of June or early in July. By the middle of September it was difficult to find a single mite present when they had been abundant before (72).

The sluggish mites had fungus filaments inside and the dead on the outside of their bodies.

BACTERIAL PARASITES OF INSECTS

A number of bacteria have been reported as causing definite diseases in insects of various kinds. The foul brood of bees due to *Bacillus larvae* White and a honeybee larval disease due to *Bacillus alvei* Chesshire & Cheyne are well-known examples. A disease of hornworm on tomato and tobacco, due to *Bacillus sphingidis* White, and septicemia of cutworms, due to *Proteus noctuarum* (White) Bergey, are other examples.

Recently a bacterium causing the milky disease in the Japanese beetle, *Popillia japonica* Newm., has been investigated and found to be effective in producing a high insect mortality (89). The causal organism is the spore-producing bacterium, *Bacillus popilliae* Dutky (16). Its relationship to the disease has been shown by inoculating several thousand healthy larvae. When healthy larvae come in contact with dead larvae left in the soil they contract the disease, which results in a high mortality. It has been found feasible to spread the disease artificially by means of spore dust derived from dead larvae, 1,500,000 larvae being used in producing over 25,000 pounds of spore dust. Diseased adults average about 500 million spores each, but larvae average about two billion spores each (32). It has recently been found that the adults, in actively migrating, carry the organism while in the process of becoming diseased, so that it is suggested that adults be collected, inoculated, and liberated to spread the disease.

Another example is a rod-shaped bacterium causing a disease of epidemic proportions on the omnivorous looper, *Sabulodes caberata* Guenée, on avocados in San Diego County, California. Insects were readily killed by inoculations with pure cultures by spraying, rubbing or injecting the insects.[2]

Only two bacterial diseases of citrus insects have so far come to the author's attention, a disease of the mealybug, *Pseudococcus citri* (Risso), in Russia, and a disease of the California red scale, *Aonidiella aurantii* (Mask). The citrus mealybug was found to be infected by red bacterium, identified as a strain of *Bacterium prodigiosum,* which was isolated and shown experimentally to be virulent for several species of *Pseudococcus* (58).[3]

A spore-forming bacterium, designated as *Bacillus C,* has been found capable of killing the California red scale in California under certain conditions (66). If we except the mealybug bacterium, this is, so far as known, the first report of a scale insect infected with a disease-producing bacterium. Although *Bacillus* C was first isolated from soil in connection with denitrification studies and was grown on chitin, cellulose and other media (68), it has since been isolated many times from the bodies of red scale in the orchard.

Bacillus C is a large (6 by 1¼ μ) Gram-positive motile rod which forms spores in the equatorial position. Its motility is usually lost after a few days of growth on ordinary media. It grows singly, in twos end to end, and in chains of four or more.

Under laboratory conditions lemon fruits heavily infested with red scale immersed in a suspension of *Bacillus* C for two to four hours developed several days later a high mortality as compared to immersion into the same medium free from this bacterium.

In some seasons in past years, especially during or after moist weather, the California red scale has been noted to have had a very high mortality from some unknown cause. In one of those seasons, fungi were suspected to be one of the controlling factors (17). It is possible that this bacterium is responsible in part for epidemics of this kind when conditions are suitable for its abundant multiplication and spread to the insects. In preliminary field trials trees sprayed with suspensions of the bacteria or dusted with mixtures of spores and clay, the mortality of adult females was twice that of the corresponding checks (66). In time, the differences between the sprayed and unsprayed trees disappeared. A species of bacterium apparently identical with the one introduced was recovered in cultures from 90% of recently killed insects tested in this experiment.

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[2] Unpublished work by Ira Ayres and D. F. Palmer.

[3] Referred to by Steinhaus (74, p. 133).

Since death of the scale is accompanied by a significant decrease of soluble-nitrate content, it is postulated that the lethal effect of the bacillus is accompanied by nitrate reduction to nitrite inside the insect. This is further indicated by the fact that certain California red scales raised on sago palm or on detached lemons and grapefruit were found on analysis to have only a trace of nitrate and were resistant to the bacillus, while those raised under natural conditions had a greater nitrate content and proved to be susceptible. Moreover, when the scales were raised on sago palm which had been given abundant nitrate, the nitrate content of the scales increased and the latter became susceptible to the bacterium (67).

Previous observations on unusual California red scale mortality have been reported. Since the California red scale in 1934 was reduced by some natural means to a very small population (17), it is now suspected that *Bacillus* C may have been one of the main factors in this natural epidemic in the orchards. A heavy mortality also of the citricola scale, *Coccus pseudomagnoliarum* (Kuw.), was noted in California at the same time. Quayle states: "mortality occurred to an unprecedented extent in the more mature scales in the winter and early spring of 1934. Control work . . . was practically entirely omitted in 1934, 1935, and 1936. No such condition approaching this had occurred since the citricola scale became a pest of citrus more than 20 years previous" (59).

In a similar reduction of California red scale in Palestine in January, 1931, a high natural mortality was found, even where no control measures had been used. Young as well as older scales shriveled and turned a dirty violet color, and the scale for that season appeared to be well controlled by some natural means (10).

DISCUSSION AND SUMMARY

Entomogenous fungi range from species that possess a high degree of parasitism to those which are little more than saprophytes.

Their effectiveness is influenced by conditions of moisture, temperature, agencies of distribution, migration of the insects and apparently by nutrition, density or other conditions of the insect host.

The highly parasitic species appear to be important in bringing about certain degree of natural control which in favorable, special situations amounts to satisfactory commercial control. Others less parasitic are of only minor importance, while still others are of doubtful value and may be little more than saprophytes.

The chinch bug fungus, *Beauveria globulifera,* as an example of common widely distributed fungi, is reported on at least 60 species of insects and has abundant wind-borne spores. The failure to attain increased mortality by artificial distribution of this fungus has been often cited as an example of what may be expected from entomogenous fungi. It may be pointed out that this result might have been expected from a fungus of this kind which has many hosts and which produces such abundance of wind-borne spores that may become widespread and reach a "saturation point" under most all conditions suitable for infection. What was found with this fungus is not necessarily a criterion by which to judge possibilities in other fungi.

The green muscardine fungus, *Metarrhizium anisopliae,* with similar characteristics as to wind-borne spores, has been used commercially for an early start of epidemics of froghoppers in Trinidad but failed to be of practical value on May beetles in Puerto Rico. There was probably a sufficient lag in spore saturation when used in the first case and more nearly saturation in the second.

In contrast, *Sorosporella* of cutworms produces neither external growth nor wind-borne spores and completes its development within the insect body, producing complete parasitism in 10 days by means of yeast-like bodies within the blood.

Among a number of fungi that parasitize the *Dialeurodes* or whitefly larvae, *Aschersonia aleyrodis* and *Aegerita webberi* are outstanding examples. Under conditions like those in parts of Florida these are spread artificially to increase their efficiency at certain seasons of non-saturation. Under certain conditions, these fungi without artificial aid appear to keep the insects at a low level and in some conditions to aid in producing commercial control. Differences in conclusions as to their value for artificial use may be due to trials under different degrees of spore saturation.

Among the fungi parasitic on scale insects, some of the most important are species of *Sphaerostilbe, Nectria, Podonectria* and *Myriangium*. In conditions of moist low-lying localities in Florida most scale insects in association with these fungi remain at a low level. It has been pointed out, however, from certain recent observations, that the efficiency of entomogenous fungi on purple scale can not be entirely judged by the phenomenal increase of this scale by spraying with bordeaux mixture or other residue-depositing fungicides. Non-fungicide residue-depositing mixtures also cause increases in purple scale. Other factors making it more favorable for their development appear to account for a considerable portion of this increase.

Species of *Aspergillus* and *Entomophthora* are discussed as important parasites on mealybugs, *Empusa* and *Entomophthora* on aphids, a species of *Spicaria* on cottony cushion scale, and one of *Rhinotrichum* and an undetermined fungus on mites.

Attention has recently been directed to bacterial parasites of insects through the success in the work with the milky disease of the Japanese beetle.

A spore-forming bacterium, known as *Bacillus* C, has recently been found capable under certain conditions of killing the California red scale. The susceptibility to attack appears to be increased greatly by the increase of nitrates in the substrate on which the insect feeds.

There appears to have been in the past a tendency to judge the possibilities in this line of investigation by the failure of some of the past experiments to show outstanding control. What is needed is to determine more definitely not only the rôle which these entomogenous fungi and bacteria play in natural control, but also to explore further the possibilities of increasing their efficiency by artificial spread in situations where the saturation point is not reached under natural conditions.

Much attention is being given to the discovery, introduction and artificial spreading of entomophagous insects and to finding ways of increasing their efficiency. The comparatively few that have given outstanding control have justified this large effort. Greater attention is needed in exploring the possibilities from similar work with various insect-destroying fungi and bacteria, and possibly insect-destroying viruses. More cooperative research by plant pathologists and entomologists should yield important results in this largely unexplored field.

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RECENT STUDIES ON INHERITANCE OF QUANTITATIVE CHARACTERS IN PLANTS

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INTRODUCTION

Quantitative characters, by definition, relate to measurable differences in degree rather than in kind, and in their inheritance usually exhibit a continuous range of variability in segregating populations. This group of hereditary characteristics is particularly susceptible to environmental influences, and it should be emphasized that it is the manner of reaction under particular conditions that is inherited and not the character itself (68, 212). As pointed out by Dobzhansky and Holz (46): "Genes produce not characters but physiological states which, through interactions with the physiological states induced by all other genes of the organism and with the environmental influences, cause the development to assume a definite course and the individual to display certain characters at a given stage of the developmental process".

An obvious distinction can be made between typical "quantitative" characters and those typically relating to "qualitative" variation, in which unlike discontinuous classes of segregants can be observed; yet it is equally evident that there is no sharp line of demarcation between the two groups, and the terms should be used merely as convenient descriptive expressions rather than as inflexible categories of classification. Mather (112, 22[illegible]) has suggested that the numerous genes determining quantitative variation, which have individual effects that are small compared to the total non-heritable fluctuations (minor, buffer or polygenes) differ from those determining qualitative variation (major, switch or oligogenes) by acting at a later stage in ontogenetic development. We would prefer, however, to suspend judgment as to differences in the nature and mode of action between these two types of genes until further evidence is available (see [illegible]).

An effort has been made in preparing this paper to review the pertinent botanical literature, mostly during the past six years

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[1] On military furlough; Lieut. (jg), [illegible]

(1937–'43), on inheritance of quantitative characters. Publications in related fields have been referred to where it was considered that they provided clues to further elucidation of this problem.

THE GENETICS OF QUANTITATIVE CHARACTER INHERITANCE

Gene Number, "Genetic Coefficients" and Restrictions on Character Recombination

The most important conception in a genetic interpretation of the continuous variability observed in families segregating for hereditary differences in quantity has been the multiple factor hypothesis (polymerism in European literature), promulgated by Nilsson-Ehle and by East. According to this interpretation, inheritance of typical quantitative characters is under the control of a number of genes or factors which are similar and relatively small in effect, usually incompletely dominant in expression and which act in a cumulative manner. Most published experimental data are in agreement with the general statistical requirements of this hypothesis, and the literature on the subject is voluminous owing primarily to the fact that the majority of species differences and characters of practical value in agriculture are under multiple genic control. Brief reviews of work on quantitative inheritance in crop plants are included in the recent text of Hayes and Immer (68) and original papers on genetic studies with cucumber (74). Medicago (12) and barley (26) will serve as recent examples to illustrate the type of data, obtained with agricultural material, that is generally considered amenable to multiple factor interpretation.

From the time of the early workers on size inheritance numerous attempts have been made to estimate the number of genes involved in a particular quantitative difference. The method used most often has been to calculate the frequency of recovery of parental classes; a high frequency was taken to indicate few genes and a low one or failure to recover the parental extremes was taken to indicate that many genes were responsible for the difference between the original types. For the most part the methods used and the conclusions reached provided little more than crude approximations of gene number, as the authors were fully aware.

"Student" (185) concluded from calculations on data involving selection experiments on oil content in maize that, for the character under consideration, there was some evidence for a gene number "at least of the order of 20–40, possibly of 200–400, and not at all likely to be of the order 5–10". Rasmusson (149) stated that the Swedish group of geneticists and plant breeders "seem to be unanimous in assuming 100–200 genes for most quantitative characters in crosses between types not too closely related". The formulae of Wright (210) for estimating the minimum number of genes and the number with manifold effects have been used by Charles and Goodwin (35) to analyze hereditary differences between the leaf characters of two species of goldenrod. They calculated that a minimum of 21 genes determined the differences between the leaf characters and that about twice as many were probably operating to control all the morphologically distinguishable characteristics between the two species.

The writer has estimated (179), by use of a formula of Wright, that not less than 12 genes are involved in determining the difference in corolla size between two species of *Nicotiana.* The prerequisite assumptions to all such estimations, however, are difficult to prove. In the *Nicotiana* cross, for example, it is apparently incorrect to assume that all the genes for small size are concentrated in the small-flowered parent, since certain extra chromosomes of this species cause enlargement of corolla parts (181).

Anderson (9) has pointed out that if the number of genes involved in quantitative character differences is as large as ordinarily postulated, then "all the multiple factor characters of an organism will be tightly linked with each other, without regard to the further restrictions imposed by frequencies of recombinations". Linkage obviously seriously restricts recombinations in the F_2, and in a particular *Nicotiana* cross, where gametic elimination, zygotic elimination and pleiotropism (defined below) also play a part, Anderson has calculated that the actual F_2 recombinations constitute less than 1/64 of the total imaginable. The writer has worked with the *Nicotiana* species and hybrids under consideration above and can add that there are also developmental restrictions to pattern recombination which, by themselves, limit the number of possible recombinations to only about two-thirds of those "imaginable". It can be shown that the genes determine relationships during development rather than absolute dimensions, so that recombinations of the length of one species with the width of another, for example, cannot be realized because of the limiting conditions imposed by relative growth.

Problems concerned with gene number, manifold or pleiotropic effects, "genetic coefficients" (defined below), developmental patterns and restrictions on character recombination are closely tied up with each other and all are intimately involved in the genetic interpretation of quantitative character inheritance. One of the long-recognized difficulties in analyzing size traits has been that many genes influence one character. The situation may be further complicated by the fact that a single gene can influence several characters by initiating one or more primary effects. This latter phenomenon is known as "pleiotropism" in the broadest sense of the term. It has been suggested by Anderson and Owenby (11) that interspecific variation is not a matter of random character differences but rather one of "harmoniously integrated tendencies" based on fundamental differentiating qualities, known as "genetic coefficients", which may produce basically similar differences throughout the organs of a plant. For example, the larger leaves, thicker stem and coarser texture of one species compared to another might be attributable to a generally larger cell size, the genetic coefficient. It was recommended (11) that species hybrids be studied in terms of genetic coefficients rather than by analyses of such complex characters as plant height or leaf length in order to obtain more direct evidence as to differences between the related germplasms. Most genetic coefficients would probably be found to be under multiple genic control. ✓

It has been indicated by Whaley (178, 200, 201, 202) that the size of determinate plant organs (flower, fruit and leaf) is dependent upon the size of the meristem from which they are produced, at least in plants with relatively indeterminate growth, as in the tomato. This observation implies that a form with larger flowers than another would also have larger fruits and probably larger leaves if the relationship is not too disturbed by the environment. While such an association may be the rule in *Lycopersicum, Cucurbita* and *Lagenaria,* it obviously does not hold for all types of plants. We have measured two closely related strains of *Nicotiana rustica,* for example, and have found that the average leaf length of strain A was 10.7 ± 0.98 inches and of strain B 13.2 ± 1.08 inches, while the average corolla length of A was 16.6 ± 1.45 mm. and of B 14.9 ± 0.83 mm. Whaley's suggestion and the more general one of genetic coefficients are undoubtedly significant, but it should be borne in mind that a given set of genes does not necessarily produce a similar recognizable external morphological effect in all plant parts, even though a basic property, such as cell size, is affected similarly throughout the organism.

The concept of multiple alleles at one locus, or possibly closely linked loci, rather than multiple genes at relatively independent loci has been useful in explaining certain hereditary phenomena of a quantitative nature. Hutchinson, Silow and others (75, 77, 166) were able to interpret differences in leaf shape in Asiatic cottons on the basis of at least five alleles at the L locus which affected sinus depth and lobe width. Hammond (62) has shown that the genes for leaf shape act by controlling correlations between growth rates in various dimensions. A system of multiple alleles has also been used to explain the results of genetic studies on bristle hairlines in *Galeopsis pubescens* (128), flower form in *Salpiglossis* (41), glume shape in *Triticum* (192) and petal flecking in *Godetia* (69).

The principle of multiple genic control is generally accepted in genetic explanations of quantitative inheritance, yet some characteristics involving hereditary differences in quantity are apparently determined by relatively few genes, and others, as plant height in maize, may be either multiple or monogenic in their inheritance. In view of these considerations and our ignorance concerning the basic difference, if any, between the genes controlling qualitative and quantitative characters, it was thought advisable, in a comprehensive review of the subject, to cite some recently published examples of hereditary traits involving size and shape which were found to be under simple genic control.

The characters dwarf habit (64, 74, 76, 87, 94), double flowers (25, 199) and differences in leaf shape (64, 147, 157) were reported in various species to be dependent on the action of single recessive genes. Other characters, such as fruit shape in *Capsicum* (121) and peach (95) and double flowers in the carnation (80), were found to be regulated in their inheritance by a single gene difference that was dominant to normal.

Lack of dominance, so characteristic of polygenic action, is less commonly observed in character differences subject to monogenic control, but has been reported, *e.g.*, for the dwarf-red type (115) and for okra-leaf shape (157) in Upland cotton. In a species with a low chromosome number and presumably relatively fewer genes, some quantitative characters might be expected to be relatively simple in their inheritance. This seems to be the case in barley which has only seven pairs of chromosomes, for in the first linkage group there are at least eight characters involving differences in quantity that give monogenic ratios (162).

It is probable that for the most part where simple Mendelian ratios occur the aberrant types represent a physiologically restricted condition. East (48, 49) has suggested that species have evolved by a progressive accumulation of small, abundant, constructive mutations with quantitative effects and that the more obvious "defective" mutations that are more frequently studied in genetics are relatively unimportant in evolution. The concept of physiologically defective genes should not be confused with that of typical quantitative factors whose action is to reduce size, for small size may be advantageous (96) and there is evidence that reductive genes play an active rôle in typical size inheritance phenomena (181).

Gene Action, Dominance, Heterosis and Skewness

Much of the recent discussion on the genetics of quantitative character inheritance has been concerned with the nature of gene action, dominance relations of alleles and interactions between genes. The conclusions that have been drawn are, for the most part, tentative, since adequate experiments that would furnish definite answers to these problems are difficult to devise and have not yet been performed. Statistics expressing averages, standard deviations and skewness have provided the main material from which genetic inductions have been made. In the footnote[2] below

[2] *Interaction*—gene action of such a nature that a certain gene substitution acts differently in combination with one genotypic background than with another. This definition is intentionally limited in scope and is essentially statistical when applied to quantitative data. The term is used frequently in genetics in the more general sense that the expression of certain (or all) characters is the end product of the combined action, *i.e.*, interaction, of several or all of the genes of the genotype; however, as a rule, it is in the more restricted meaning, as defined above, that the word "interaction" has been used in publications on quantitative inheritance.

Arithmetic action—each gene substitution adds or subtracts its contribution to that of the residual genotype. If the absolute amount added to the phenotype is the same regardless of the other genes present the gene in question would be considered as having independent arithmetic action.

Additive action—arithmetically accumulative and usually implying lack of interaction.

Geometric action—each gene substitution multiplies or divides the residual genotypic value by a certain amount. The absolute phenotypic contribution of the gene would vary according to the magnitude of the residual genotype, we have listed, as an aid to the discussion to follow, brief explanations of some of the terms encountered frequently in papers on quantitative inheritance.

The results obtained from crosses between types differing in quantitative characters have been extremely varied, owing undoubtedly to complex anatomical and physiological conditions as well as to those of a genetic nature. Some workers have attempted to examine their data for evidence indicating whether the gene action is more nearly arithmetic or geometric in effect, though neither scheme is expected to apply strictly for all genes or for all organisms. "It seems likely, on the whole, that the genes determining any particular size difference would be found, if isolated singly, to be rather diverse in nature of action and in synergetic relations as well as in magnitude of effect and degree of dominance. But the isolation of monogenic differences is generally so difficult in the case of quantitative characters that it is often practicable only to determine which of commonly proposed simple schemes of inheritance comes nearest to compatibility with the data" (36).

One type of result obtained frequently in studies on size inheritance is characterized by the F_1 mean approaching the geometric mean between the parents and a positively skewed frequency distribution for F_2 measurements. These data have been interpreted by some to indicate a probable geometric gene action (34, 36, 103, 104, 142, 143, 144, 145, 170, 179, 181); others have suggested (96) that they could be explained mainly on the basis of arithmetic effects with partial dominance of genes for small size, and it is also possible that the metrical bias observed has no genetical significance (111).

The arguments advanced by Lindstrom (96) for favoring the arithmetic scheme with partial dominance of genes for small size were based mainly on evidence for a decrease in the mean variability and positive skewness in a $4n$ F_2 compared to a corresponding $2n$ for measurements on fruit size in tomato. It can be shown,

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but if it acts as a fixed multiplier the gene would be considered independent and geometric in action.

Positive skewness—a type of departure from a normal frequency distribution curve characterized by the median being to the left of the mean and a "long tail" to the right. A skewed distribution in the opposite direction is called negative.

Metrical bias—skewness in a + or − direction caused by an "inherent" relation between the scale of measurement and phenotypic expression. It may or may not have genetical significance and can be removed by an appropriate transformation of scale (53, 111).

however, that these same results would be expected if the genes acted in a geometric manner without dominance or interaction, and, furthermore, if many genes determine fruit size (as seems to be generally agreed is the case) one would not expect to find pronounced skewness due to dominance in a relatively small sample population.

We are inclined to favor a general geometric interpretation, at least for the results with fruit size in tomatoes and squash and corolla size in *Nicotiana,* because it has a reasonable morphological and physiological basis (104, 171) and because the empirical statistics are in remarkably close agreement with theoretical expectation (36, 103, 181). The suggestion that hereditary factors controlling size, growth or quantity may have geometric, multiplicative, percentage or exponential effects is not a new idea; it can be found in earlier publications, notably those of Galton (1879), East (1913), Groth (1914), Zeleny (1920) and Wright (1922). Evidence from morphology indicates (71, 104, 173) that gene substitutions act by controlling such properties as cell size, number of cell divisions or the integrated growth process and, therefore, might be expected to have a general multiplicative effect on the size of a mature organ or organism. However, any measured quantitative character is, in itself, a complex biological entity subject during development to a variety of stresses, and a gene substitution affecting a particular histological condition should not necessarily be expected to produce a strictly geometric effect on the end product. The results of experiments on quantitative inheritance in *Zea mays,* for example, are in general more amenable to an arithmetic interpretation; and, from the great variety of data with other plants and animals, it appears unlikely that a simple consistent type of gene action can apply to all.

Data on corolla size in a series of interspecific polyploid hybrids of *Nicotiana* (181) afforded an illustration of both a simple and a complex relationship in size inheritance phenomena. It was found that each genome substitution had an apparently simple geometric effect on corolla size and that polyploidy acted as a multiplier only on the size of the tubular part of the corolla and not on the length of the limb. The polyploidy effect is essentially a histological problem but it does indicate that if a gene substitution affected only cell size it would not necessarily produce strictly geometric effects on a particular quantitative character. On the other hand, typical "size genes" probably act in a more harmonious manner, controlling coordinated growth processes rather than one property. In the above experiments a modified multiplicative scheme, in which the numerical value of each locus was considered to be equal to the geometric mean of the alleles involved, was adopted as a consistent, workable system for explaining the genetic results in both diploids and polyploids. It is probable that the multiplying values of most of the genes producing increases in size are around 1.1 or 1.2 and those causing decreases are around 0.8 or 0.9.

From the extensive data on first generation hybrids between types that differ in quantitative characters it is evident that the F_1 mean may exceed the value of the larger parent; be less than that of the smaller (168); or have almost any intermediate value. The latter result is most commonly observed in studies on size inheritance and has led to the belief that, in general, most genes controlling quantitative inheritance are incompletely dominant in phenotypic expression.

Hybrid vigor or plus heterosis, in which the F_1 is larger than either parent or at least exceeds the mean between the two parents, is also a frequently observed phenomenon and, in the currently most accepted genetic interpretation first proposed by Jones, is explained on the basis of an accumulation (in the heterozygous condition) of dominant genes favorable for growth. Dominance is assumed whether heterosis is considered "normal" vigor achieved through heterozygosity by the hiding of deleterious recessives, as suggested by Collins, or "supernormal" growth caused by an "excessive" accumulation of advantageous genes. The convergent improvement method for corn breeding outlined by Richey is based upon and affords a means for testing this dominance hypothesis; and the results of recent experiments (68, 221) tend to confirm the theory that at least part of the heterotic effect can be explained on this basis. It is reasonable to consider heterosis as one type of result in the general category of quantitative inheritance, and it appears likely, therefore, that the genes affecting quantitative characters, like those producing qualitative effects, may manifest different degrees of dominance.

East (50) proposed another genetic explanation for heterosis in which the increased vigor was attributed in large measure to the accumulative action of multiple alleles. He assumed that the genes concerned with heterosis lack dominance in the classical sense and control "non-defective" physiological reactions. Mutations, that occur frequently at the loci involved, produce alleles with slightly different physiological action. When certain of the divergent alleles are combined in the heterozygous condition they act in a complementary manner, thus producing a more efficient physiological condition which finds phenotypical expression as hybrid vigor.

R. A. Fisher and certain British geneticists (53, 78, 111) have maintained that heterosis is due to a progressive selection for dominant + genes having taken place and that in unselected material dominant genes that act on growth in a + and in a – direction would be present in a genotype in approximately equal numbers. In regard to the phenomenon of dominance the conclusions of Silow (166), based on observations pertaining to the genetic analysis of leaf shape in cotton, are of interest. He is of the opinion that dominance is best regarded as a function of allele interaction, dependent upon the magnitude of the gene's effect relative to the possible range in expression of the character affected, as well as upon other genes working toward the same result. The system may be conceived as an interplay of "threshold" and "saturation" effects.

A recent comprehensive review of the subject of heterosis has been prepared by Whaley (203) who has discussed the various explanatory hypotheses. In conclusion, the solution of the problem of heterosis, like that of the more general one of quantitative inheritance, awaits the presentation of more conclusive experimental evidence in support of any one of the explanations offered.

Mather (108, 110, 111, 112, 113) and Wigan (205, 206) have published papers recently on the rôle of quantitative inheritance in relation to selection and evolution. The general concepts presented have been summarized in a review article by Mather (111) and may be outlined in brief as follows: The greater part of species differentiation is quantitative or polygenic, *i.e.*, controlled by many genes with similar action whose individual effects are small compared to the total non-heritable fluctuation, and variability is maintained at a high level as an essential for prospective adaptive and evolutionary change. By the action of natural selection, so-called "balanced" combinations of polygenes are built up within each chromosome. Systems so produced will be characterized by "close adaption to the optimum, great variability storage and slow variability release" (111), and may be pictured genetically as composed of dominant genes acting to increase the expression of a character distributed throughout the chromosome and combined (preferably by close linkage in the repulsion phase) with an approximately equal number of genes acting to decrease the expression of the character. Variability is released by segregation and recombination. Balance is disturbed in recombinations, and the resulting phenotypic variability is acted upon by selection. The response to selection and random elimination of alleles reduced variability which, however, is replenished by mutation.

The shape of the frequency distribution curve has been used by some as a basis for interpreting their results on quantitative inheritance. Caution should be exercised, however, in interpreting skewness, for different causes, equally likely *a priori* and difficult to distinguish, may produce skewness in either direction; furthermore, a normal distribution may indicate balance between opposing tendencies rather than freedom from them.

Positive skewness may be caused by a predominance of dominant genes for decreasing size, an approach to a physiological lower limit of phenotypic expression, interactions of such a nature that each gene acting to reduce size has progressively less effect the more there are acting in the same direction, or by geometric action of the genes. Negative skewness may be caused by a preponderance of dominant genes for increasing size, an approach to a physiological upper limit of phenotypic expression, or by interactions of such a nature that each gene acting to increase size has progressively less effect the more there are acting in the same direction (149).

Genes obviously interact, in a general sense, to produce characters, and some with major effects (see 167) are known to interact in the more specific sense defined in the footnote on page 354; yet there is no clear evidence that this latter type of interaction occurs between typical multiple genes controlling the inheritance of quantitative characters. Mather (111) has pointed out that the interactions reported in *Pisum* (150) and *Lycopersicum* (40) could be due to metrical bias; nevertheless, he is of the opinion, and most investigators would no doubt agree, that until more information is available it would be discreet to suppose that any type of interaction shown by genes with major effects may also be found among the so-called polygenes. Powers (141) concluded that there was no general rule for interaction between genes determining quantitative characters, but he observed that genes favorable to high weight of seed per plant in *Hordeum* gave greater increases over their alleles in combination with non-allelic genes for higher yield than with those for lower yield.

The concepts of directional interaction, causing skewness in one direction, and of physiological limits should be distinguished in theory, for the former is genetic and could be independent of the magnitude of the phenotype, while the latter is physiological, directly dependent on it, and would be found only where certain bounds were exceeded. Skewness due to physiological limits might occur in a homozygous line and in this way would be distinguishable from that due to dominant genes which necessarily would be the result of segregation in heterozygotes (149). Skewness would be accentuated if a character were determined by relatively few dominant genes or if some of the genes had major effects.

If a wide enough range of phenotypic values were available, physiological limits might conceivably be approached in both directions. We apparently have an example of this in some unpublished data collected in the Division of Tobacco Investigations on selection for low and for high nicotine content in *Nicotiana tabacum*. Frequency curves for alkaloid content in presumably homozygous low nicotine selections that had on an average around 0.5% of the alkaloid in dried leaves, were positively skewed, while the distributions in high nicotine selections (4.5–5.0%) were negatively skewed. A sensible interpretation of the results with the selections for high nicotine would be that there is a physiological upper limit to the amount of nicotine that can be accumulated within the tobacco leaf which, in the strains under consideration, is around 4.5 to 5.0 per cent. The positive skewness in low nicotine selections may be attributable to an inherent essentiality of nicotine, however low in concentration, to life processes in the tobacco plant.

Another example of data exhibiting + skewness at the lower end of a scale and – skewness at the upper is found in the distribution curves for "total index values" in comparisons between species in the genera *Tradescantia* (5), *Solidago* (58), *Iris* (153) and *Aster* (196). The indices were only arbitrary quantitative measures derived by crudely combining a group of observed characters by which the species are differentiated, but the consistent shape of the distribution curves may indicate that the combined characteristics accumulated within a natural species do, in some way, approach morphological or physiological limits of development along a particular evolutionary line.

The skewness of metrical bias may be in either direction, depending upon the relation of the scale of measurement "to the unknown 'scale', on which the organisms' physiological processes work" (111), as log. to antilog.; but its effect would be consistently in one direction for the data on a particular quantitative character. Negative skewness in frequency distributions for measurements on plant height in barley was interpreted as due mainly or wholly to metrical bias attributable to environmental influence (53).

Other conditions that may affect skewness in either direction are interactions that are not unidirectional (*e.g.,* different conditions of epistasis and complementary gene action), the amount and type of linkage, and differences in the proportional contribution of the common residual genotype to that of segregating genes in making up the total value of the phenotype.

Methods of Analysis

The nature of quantitative character inheritance makes it necessary to record experimental results in some form of measurement and, for final analysis, to treat the data biometrically. In addition to the use of generally accepted methods of experimental design (52) and statistical analysis (51), formulae that refer more specifically to quantitative inheritance have been developed and used. A few that might be mentioned here are the use of third degree statistics in analyzing skewness (53), methods for separating environmental from genetic variation (209, 36, 140, 145), estimation of the number of gene substitutions determining an hereditary difference (210, 35), a "mathematical notation . . . for expressing the . . . morphological hiatus between two species" (11), criteria for distinguishing between the results of geometric *vs.* arithmetic gene action (36), and a method for determining the degree of influence of size factors with general and with local effects (208).

Other analytical methods, more cytogenetic than mathematical, have been developed for the purpose of investigating quantitative inheritance by breaking down the usual continuous segregation into a discontinuous one. This can be accomplished by utilizing inversions, reductions or additions in chromosome number, and linkages with qualitative characters. These methods are not expected to reveal the effect of monogenic differences, but rather, to indicate the action of groups of genes located in certain chromosomes or parts of chromosomes.

Since the early work of Sax, Lindstrom and others on linkages between qualitative and quantitative characters, other associations of this sort with plant material have been reported more recently in the literature. In tomato, linkages were found between time of fruit ripening and three genes on the first chromosome (40), between two marker genes and both locule number and fruit size (142, 143), and between plant height and fruit weight (104). In addition, genetic relationships between genes affecting corolla size and color in certain species of *Nicotiana* were reported (179), and a number of similar linkages found in barley have been summarized (162).

The use of inversions as a possible method for locating favorable genes in maize has been proposed (47). In brief, the procedure consists of crossing a stock that contains an inversion tagged with a recessive color gene, *e.g.*, to an inbred line having the corresponding dominant, and comparing the two color classes in an F_2 or backcross population with respect to certain quantitative characters. Single crossovers in the inverted region would be eliminated because of deficiencies, so that for all the genes, except those in this region, the two classes would be statistically equivalent and any difference in yield would be due to "yield genes" located in that part of the chromosome involved in the inversion.

Sprague (183) tested this method in a cross between an inbred dent corn carrying the *Pr* allele and a stock homozygous for a fifth chromosome inversion that included the *pr* locus. He reported significant differences in yield per plot and weight per 500 seeds between *pr* (red) and *Pr* (purple) groups in a segregating backcross population. The favorable factors were contributed by the dent corn inbred parent. "No significant differences were found for plant or ear height, number of ears per plot, kernel row number or moisture content".

Numerically aberrant chromosomal types, in which one or a pair of chromosomes is involved, have been used to demonstrate the effect of the constituent genes on morphological characters. Single extra chromosomes produce obvious quantitative effects on plant parts in *Datura* (177) and *Nicotiana* (56, 181), for example; and forms lacking in one or a pair of chromosomes have been shown in *Nicotiana tabacum* (38) and *Triticum vulgare* (164, 165), respectively, to deviate markedly in appearance from those with a normal chromosomal complement.

Positive results, demonstrating the existence of genes affecting quantitative characters in any chromosome that can be tested, are to be expected, since such genes are presumably multitudinous and well distributed throughout the complement.

EVIDENCE ON QUANTITATIVE INHERITANCE FROM MORPHOLOGICAL AND PHYSIOLOGICAL INVESTIGATIONS

Relative Growth and Inheritance of Form

Recent advances toward a better understanding of the nature of hereditary growth differences in plants have been made through studies on relative growth in regard to both internal and external development. Sinnott and his associates have demonstrated the value of shifting the emphasis of genetical studies from end products to relationships during ontogeny. The term morphogenesis has been used to refer to the field of investigation pertaining to the origin and development of form.

One method used to investigate this problem has been to record measurements of dimensions that affect the shape of an organ, as length and width, from early primordia to maturity, and to compare hereditary shape variants on the basis of relative growth curves constructed from data so obtained. Sinnott (171) reported, from an analysis of this sort, that "In *Lagenaria,* the earliest ovary primordia are all much alike and the marked differences in fruit shape which later arise are due to differential growth of the dimensions throughout the whole course of development. In the bottle gourd, for example, growth in width is slightly but steadily more rapid than growth in length, so that the fruit becomes progressively flatter as it grows larger. In the Hercules club, on the other hand, length growth is constantly more rapid than width growth, so that fruit shape becomes more and more elongate". The relative growth rate of the dimensions remained constant throughout development so that when plotted graphically (log. length by log. width) a straight line was formed; and the slope of this line was the significant hereditary constant determining shape.

In other species, such as *Cucurbita Pepo* (169), there was little difference in the relative growth constant in types with different growth shapes; but differences were established in the primordia very early in development and were maintained to maturity. Similarly, in Houghtaling's (71) investigations on tomato fruits, it was shown that shape differences were established in the ovary primordium before flowering as a result of differential growth rates between polar and equatorial dimensions; and subsequent relative growth rates were the same for all forms (see also 104 and 213).

These developmental relationships may be expressed mathematically by using Huxley's formula for relative (heterogonic or allometric) growth, namely, $y = bx^k$. In analyzing shape differences x and y have been taken as two dimensions of an organ; k is the constant ratio between their growth rates (the slope of the line); and b is a constant representing an initial relation between the dimensions.

A study of relative growth rates has been found useful in recent investigations on the inheritance of leaf shape in golden-rod (59), *Aster* (43), *Nasturtium* (204), cotton and other genera (62); and on the shape of the ovary wall in *Iris* (154). Commonly there is a sudden break in the direction of the relative growth curve which in *Solidago* (59) was found to be correlated with cessation of cell division and initiation of rapid cell enlargement.

Histological Conditions Associated with the Development of Size and Shape

Research on correlations between differences in size and shape of organs during development and their constituent cells is a field of investigation that has only recently begun to be explored. Reviewing the evidence from studies on fruit size in the Cucurbitaceae regarding relations between the size of cell and organ, Sinnott (173) concluded that "it is the organ or body which shows the more simple and unitary growth program in the attainment of a specific size, and the cells of which the organ is composed display a much greater diversity in size, number and rate of change". This conclusion was based on four groups of evidence that may be outlined as follows:

a) The fruit develops according to a regular growth cycle that is very similar in all types despite differences in relations between fruit size and the size and number of the cells. The general histological features of fruit development in cucurbits are that tissue growth takes place at first chiefly by cell multiplication accompanied by a slow increase in cell size; and, after a specific cell size is reached, division ceases and all further growth of this tissue is by cell expansion. This is also essentially the situation in the development of tomato fruits (71, 104) and the leaf of goldenrod (59).

b) Growth of the fruit proceeds at a constant logarithmic rate, even when there is an abrupt shift from cell multiplication to increase by cell expansion.

c) Growth of the plant proceeds at a constant logarithmic rate though the volume of the meristematic cells decreases (200, 201).

d) From measurements on growth in root tips it has been shown that after division ceases in a given lineage, elongation of each cell is at first more rapid at its basal (proximal) end and later at its apical one. "It is thus brought about not by a uniform expansion of one cell after another in a series toward the apex, but rather by a "wave" of elongation which proceeds apically and affects first one end of each cell and then the other. This is not what one would expect to happen if growth of the organ were the result of growth of relatively independent cellular units" (173).

Bindloss (28) found no positive relation between meristematic cell length and mature stem length in a developmental study of tall and dwarf races of tomato; but the former had a longer meristematic region, thus accounting for greater height by making a greater number of cell generations possible. The length of panicles in larger forms of rice compared to dwarf types was ascribed to more active cell divisions (133). No difference in cell size was noted. Abbe and Phinney (1) reported that dwarf maize plants (d_1d_1 in constitution) were shorter than normals, owing to the fact that cell division and enlargement had proceeded more slowly.

It is evident that differences in cell size or number or both may be found in plants that differ in organ size.

Developmental anatomical studies have been fruitful in yielding information on the morphological basis for hereditary differences in shape. It has been shown in the cucurbits that the determinative basis of form is independent of organ size and thus, according to Sinnott (173), of the number and size of the cells that compose the organ. There was no essential difference in the shape of component cells of races with different shaped fruits. It would appear, therefore, that the relation between the number of cell divisions in one plane compared to those in another would be the controlling factor in determining shape differences.

In confirmation of this hypothesis, Sinnott (173) found from developmental studies that in cucurbit fruits where length and width increase at an equal rate mitoses are equally abundant at all angles, whereas in the club gourd where length grows more rapidly than width there is an excess of figures which make a relatively small angle with the axis. The genotype of the organism appears to have a directive influence by causing a coordinated swinging of the angle of the mitotic figure, cell plate and new cell wall into the required position (173).

Hammond (62) found that in cotton "Shape differences between broad and narrow leaves are essentially due to differences in relative numbers of cells in the length and width planes". The relatively longer and narrower leaf of *Solidago sempervirens* compared to *S. rugosa* was reported (59) to be "due to less pronounced marginal cell division, to greater elongation of the cells parallel to the midrib, and to prolonged, polarized cell division in the basal, petiolar portion". It was shown that differences in leaf shape between two species of *Aster* were attributable partly to limitations on the number and direction of cell divisions and the extent of cell enlargement (43).

Leaf pattern in a cross between two species of *Tropaeolum* (204) was found to be determined very early in development by differential cell division in such a way that in regions where multiplication was more rapid, small cells with greater expansion potencies were laid down. Final shape was determined by the extent to which cell enlargement developed the potential pattern of the juvenile leaf, and was controlled mainly by two genes with cumulative effects.

Differences in the relative growth rate of certain regions of *Iris* ovaries were reported to be dependent chiefly on differences in the relative rate of cell division (155, 156).

The effect of polyploidy on size and shape should be mentioned here, though no attempt to review the literature on this subject will be made. Generally, an increase in chromosome number causes an increase in cell size which frequently results in larger plants and organs, provided an upper limit of tolerance is not surpassed. The main effect of polyploidy on shape is to bring about proportionately greater increases in width than in length so that the organs are relatively wider and thicker in the types with higher numbers of chromosomes (175, 180, 181, 215).

Experimental Evidence Indicating Possible Physiological Bases for Quantitative Inheritance

It is generally recognized that the visible expression of gene action is the manifestation of fundamental physiological processes which, in turn, rest ultimately on a physico-chemical basis. Though we know little of the complex series of chemical and physical changes involved in the development of quantitative traits, a few experiments have been reported recently which deal with the physiological basis of hereditary growth differences. These investigations were concerned chiefly with inherited differences in the production of growth-regulatory substances and with hereditary variation in plant nutrition. Any statements relating these experimental results to the physiological basis for quantitative inheritance should be qualified, however, for it is possible that no such correlation exists, though the speculation is attractive.

A number of genetic dwarf types in maize were found by Overbeek (136) to produce auxin at a lower rate than their normal sibs, and tissues of the recessive known as "nana dwarf" inactivated auxin more rapidly than tissues of normal plants. Goodwin (57, 59) observed, from a study of the amount of auxin diffused from the leaf bases of two species of goldenrod, that more was obtainable from *Solidago sempervirens* than from *S. rugosa.* He inferred that certain genetic differences in leaf size, leaf shape and type of inflorescence could be attributed to differences in the amount of auxin in each species. Two species of *Aster* that differed in mode of branching were studied by Delisle (42) who concluded from the data "that the branching habit in these species of *Aster* is largely correlated with the amount of auxin produced in the terminal bud and to a lesser extent with that produced in the young leaves".

It was reported by Lehmann (91, 92, 93) that inhibited hybrids of *Epilobium* had less growth substance in the apical bud than normal growing plants, and he suggested that their limited development might be explained on this basis. Heteroauxin was applied to the stem tips of seedlings of *Epilobium* dwarf hybrids (163) and the treatment was observed to cause increased cell elongation and inter-nodal length, but it decreased the size of developing leaves. Michaelis (118, 120) is vigorously opposed to Lehmann's interpretation that the inhibition phenomenon in crosses between species of *Epilobium* can be explained adequately on the basis of a limiting amount of growth hormone. Went and Thimann (195) have suggested the possibility that sensitivity to growth hormone might be determined by the genes, and auxin production by the cytoplasm.

The facts that there is evidence of inactivation of indoleacetic acid in corollas of *Nicotiana Langsdorffii* (132) and that certain extra chromosomes of this species cause a reduction in flower size (181) has suggested the explanation that these chromosomes may contain genes for small size whose physiological action is to inactivate growth hormone.

The physiology of heterosis is a field which has been actively investigated during the past few years, and a number of papers, both polemical and experimental, have been written on the subject. The work has been confined almost entirely to studies on tomatoes and maize. Whaley (203) has recently reviewed the literature on this phase of heterosis so that no detailed account will be presented here. It is sufficient to say that the only unifying conclusion that can be drawn from the varied but not necessarily contradictory collection of data, appears to be that heterosis involves essentially an increase in growth rate which may take place at any stage of development, with the possible exception of the grand period of growth, from very early (four days after fertilization (39)) to very late (just before maturity (201)), depending upon the genotype under consideration.

The auxin content of maize kernels from plants with varying heterotic vigor has been investigated by Avery and co-workers. They reported that F_1 hybrids were intermediate between their inbred parents in content of growth hormone per gram of endosperm (19) and that there was no apparent relation between the vegetative vigor of hybrids and the amount of auxin stored in the kernels produced by them. It was pointed out, however, that there might be a correlation between auxin content in the vegetative tissue and hybrid vigor, but that research on this problem awaits development of adequate methods for extraction of auxin from green tissues.

Robbins (158, 160) reported that an extract of grains of heterotic hybrid corn produced a greater growth-promoting effect on *Phy-*

comyces than that of either inbred parent. The effect was ascribed to the presence of an unidentified growth-promoting substance called, for convenience, factor *Z* which is present in higher concentrations in the hybrids. It was suggested that the growth of both parents and hybrids may be limited by the quantity of factor *Z* which each synthesizes.

In another paper Robbins (159) reported the results of growing excised roots of two inbred lines of tomato and their heterotic F_1 hybrid in synthetic solution cultures supplemented with various combinations of the growth substances thiamine, pyridoxine (vitamin 6), nicotinamide and thiazole. The F_1 roots grew more rapidly and produced more dry matter than those of either parent except in the solution supplemented with thiazole, in which one parent (Red Currant) grew best. The three kinds of roots responded differently to the different solution cultures and the results were encouraging to the speculation that heterosis might depend on the ability of heterotic plants to synthesize or to utilize growth substances more efficiently than inbred lines.

The study of hereditary differences in the ability of plants to utilize various sources of nutrition offers another method of approach for investigating the physiological basis of size inheritance. There is general recognition of the fact that different species, and to a lesser extent varieties and strains, differ in their response to nutrient conditions, though few investigations have been reported which deal with this matter in any detail.

Harvey (65) grew corn inbred strains and hybrids in aqueous mineral solutions supplied with various nitrogen sources and found that the inbreds showed differential nutritional responses which were attributed to inherent genetic differences in the strains and were transmitted to hybrid offspring. He also grew strains of tomatoes on three deficient nutritional solutions (low N, low P and minus K) and compared the growth with that on a full nutrient solution. Significant differential growth was made by strains on each nutritional deficiency, and the differential response to potassium levels was inherited.

Burkholder and McVeigh (33) observed differential growth of inbred lines and hybrids of maize in response to higher increments of nitrogen which was attributed to differential efficiency in synthesizing protein. Certain soybean varieties from Manchuria when grown in soil with low available iron showed (194) deficiency symptoms of severe chlorosis, while other varieties under the same conditions made normal growth. This inefficiency in iron utilization was conditioned by a single recessive gene. Aerial tissues of plants homozygous for the inefficient gene had a relatively higher pH, lower soluble iron, higher total iron and lower potassium content than normal. It was suggested that the "gene may condition lower potassium concentration which produces higher pH of cell sap and thereby causes the solubility of iron to be decreased" (194).

It is not unreasonable to conjecture that some of the hereditary differences in size, yield, *etc.*, may be the visible manifestation of gene action which affects physiological processes controlling differential efficiency in the utilization of available nutrition.

The recent work of Beadle and Tatum (23, 188) on *Neurospora* is of particular interest as experimental proof of hereditary differences in growth due to genetically controlled biochemical reactions. Mutant strains, induced by x-rays, were unable to carry out specific biochemical processes essential to the growth of the fungus. In one, the ability to synthesize the thiazole half of the vitamin B_1 molecule was lost; a second was wholly or largely unable to produce vitamin B_6; and in a third strain paraaminobenzoic acid was not synthesized. The latter two strains each differed from normal by a single gene. The deficient strains could be made to grow normally if the missing growth substance was supplied to the culture medium. The results have been summarized (226) as follows: "Mutants so far obtained include strains deficient in the syntheses of the known vitamins, thiamin, thiazole, nicotinic acid, pantothenic acid, paraaminobenzoic acid, and pyridoxin, and the amino acids methionine, lysine, arginine, leucine, and tryptophane. Strains have also been obtained which appear to require certain purines or pyrimidines, but the requirements for these have not been completely worked out as yet".

THE RÔLE OF THE CYTOPLASM IN QUANTITATIVE CHARACTER INHERITANCE

Though the many genes presumably responsible for continuous quantitative variations have not been isolated and studied individually, the bulk of evidence from segregations, similarity of reciprocal hybrids (48), linkages with qualitative characters and effects of individual chromosomes (see discussion above) should provide adequate grounds for concluding that the factors controlling most hereditary differences capable of experimental analysis are intranuclear, chromosomal and particulate.

Nevertheless, a substantial amount of evidence has been accumulated that the cytoplasm in certain genera plays a differential rôle in heredity (32, 93, 118–120, 134, 151, 197, 222). The characters most liable to be affected by differences in cytoplasmic background are pollen sterility and those of a quantitative nature, as habit of growth and size and shape of vegetative and reproductive organs. The general results have been to demonstrate differences in reciprocal hybrids of such a character that when a certain species or biotype was used as the maternal parent in a cross, and thereby furnished the cytoplasm, the hybrids were inhibited in development, while the reciprocal F_1's were well developed.

It has been concluded, therefore, that certain cytoplasmic substrata exert an influence on hereditary traits which is observed largely as an inhibition of the full expression of particular quantitative characteristics.

In the more recent work of Michaelis (120) he reported results from crossing 38 races of *Epilobium hirsutum* reciprocally with a race from Jena. All crosses with Jena as the male had normal vigor, but in Jena plasma a continuous series of increasing abnormalities was observed in the F_1, depending on the race used as male. Michaelis arbitrarily grouped the hybrids into three classes. The first showed only slight reciprocal differences and the third was very dwarfed and never flowered. The second group of hybrids in Jena plasma were described as dwarfed with reduced leaf size and internode length, various types of leaf spotting, reduced anthocyanin and increased chlorophyll content, reduced flower size and partial or total pollen sterility. The degree of growth inhibition was interpreted as due primarily to the reaction of the genotype of the pollen parent to the inhibiting characteristic of the Jena cytoplasm.

In the F_1 cross of Jena (female) × München race (male), variants from the normal type of growth inhibition were found, indicating that the München race segregated for genes susceptible to inhibition by the Jena cytoplasm. In the cross of Jena (female) with another race, Wien, the various F_2 and backcross populations indicated the presence of at least three pairs of nuclear genes for dwarfing, two for abnormal flower size and three for abnormalities of leaf shape. These genes were independent in segregation but acted together in Jena plasma to produce the abnormalities.

The evidence indicates, therefore, that at least in a certain few genera, as *Oenothera* (151), *Streptocarpus* (134) and *Epilobium,* the cytoplasm of one species may inhibit development of the genotype of related species; and, furthermore, that in the latter genus the effect of independent nuclear genes susceptible to plasma-inhibiting influences can be detected.

SUMMARY

Quantitative character inheritance is of interest and importance in genetics, plant breeding and evolution because most characteristics of practical value in agriculture and the majority of differences between species show this type of heredity. According to the generally accepted hypothesis, typical quantitative characters are under the control of a large number of genes or factors which are similar and relatively small in effect, non-dominant in expression, and which act in a cumulative manner. Estimates of the number of genes determining the inheritance of a particular measure of quantity have usually been placed at more than 10 and up to possibly as many as 100 to 200. It is to be expected that, because of the many genes affecting each quantitative character, close linkages would occur which, together with other conditions, would impose severe restrictions on the range of character recombinations in hybrid populations.

The concepts of genes producing manifold effects by controlling primary developmental processes or of a battery of genes determining "genetic coefficients" with either local or general visible manifestation have served to emphasize both additional complexities and possible simplifications in the analysis of quantitative inheritance.

In some experimental material, genetic evidence, supported by morphological observations, has shown that gene substitutions affecting size are more nearly geometric than arithmetic in effect. However, a strict and simple geometric interpretation is not compatible with and cannot be expected to apply to all data on size inheritance.

√ Hybrid vigor or plus heterosis, one type of result in quantitative inheritance, is probably explained best as due to the action of dominant genes favorable for growth which have been accumulated by selection in inbred lines and which in an F_1 are present, in the heterozygous condition, in maximum numbers.

Skewness in the frequency distribution of measurements of quantity may be due to dominance, the nature of gene action, physiological limits to phenotypic expression, interactions of various sorts, and metrical bias. It is difficult to distinguish between these different causes so that caution should be exercised in interpreting the genetic meaning of departures from a normal distribution curve.

A cytogenetic approach to the study of quantitative inheritance is being used increasingly in addition to established and improved methods of statistical analysis. The utilization of inversions and of alterations in chromosome number offer promise for more extensive study in the future.

Developmental investigations on size and shape of plant organs have given us further insight into the more fundamental aspects of their inheritance. It appears that a difference in organ size may be correlated with one in cell size but more often with a difference in number of cells. Similarly, the relation between number of cell divisions and amount of cell expansion in one plane compared to another is the controlling factor in determining differences in shape.

The ultimate physiological or physico-chemical basis for inheritance of any quantitative character is unknown, but clues are suggested by certain recent results demonstrating inherited differences in the production of growth-regulatory substances, in the utilization of plant nutrition, and in the ability to synthesize materials vital to the life processes.

Most genetical studies show that the factors responsible for continuous quantitative variations, while difficult to isolate singly, are nevertheless intranuclear, chromosomal and particulate. Yet there is evidence that in a few genera the cytoplasm plays a differential rôle in heredity by limiting the development of hybrids with certain cytoplasmic substrata. The effect is shown largely by the inhibition of particular quantitative characters.

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RADIATION AND PLANT RESPIRATION

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INTRODUCTION

Over a period of nearly a century the many investigations which have dealt with the influence of radiation upon respiration and fermentation of plants have given rise to a mass of contradictory and confusing data. The situation is doubtless to be attributed, in large part, to the inadequate facilities for control and measurement of environmental factors, such as temperature and radiation, which have generally been available. In other cases the analytical methods used have not been sufficiently precise for the determination of small effects. In addition, there is evidence in some work that apparently minor variations in the biological materials and experimental conditions employed may have greatly influenced the results obtained.

The present review is little more than an attempt to assemble the scattered literature bearing on the subject of radiation and plant respiration. An extended theoretical discussion of the mechanisms involved would not seem to be justified by the experimental evidence so far available, nor will it be feasible to give more than passing mention to many collateral fields of knowledge which eventually may prove to contribute to an explanation of these mechanisms. Consideration of photodynamic effects and of the reversal of chemical inhibition of respiration by light has not been included. For previous reviews of various aspects of the literature, the reader is referred to (49, 61, 156, 179, 245, 326).

The influence of radiation upon plant respiration is of interest not alone in its bearing on our understanding of respiration itself and of the effects of radiation upon plants, but also because of the importance of evaluating the rôle of this factor in experiments dealing with other aspects of respiration or with photosynthesis.

It will not be attempted here to formulate a definition of respiration from the standpoint of the vital economy of the organism. Indeed, with our present crude experimental technics it is often impossible to distinguish respiration from other processes which may be functionally quite unrelated to it. Theoretically, respiration may be measured by changes in the composition of the respiring material or by changes in the environment. Actually, the latter methods (usually employing alterations in oxygen and/or carbon dioxide content of the environment) are far more practicable and convenient and have been used almost without exception in the experiments herein reviewed.

In the following discussion the literature has been arranged primarily with respect to the kinds of radiation and the types of biological materials studied, rather than in chronological sequence. As a matter of convenience a quite arbitrary classification has been adopted.

EFFECTS OF VISIBLE RADIATION ON RESPIRATION

Since the overall respiratory reaction is, generally speaking, just the reverse of the photosynthetic reaction, direct evidence of alterations in respiration, due to light, can be obtained only with material naturally incapable of photosynthesis or in which photosynthesis is suppressed by suitable experimental treatment. Indirect evidence along a number of lines may be derived, however, from photosynthetic material.

It should be borne in mind that in many of the studies to be discussed in this section it is probable, or even certain, that infra-red, and in some cases also ultra-violet, was included in the radiation employed. The sources of radiation and types of filters used will be indicated wherever such information is available.

Non-Chlorophyllous Plants and Tissues

Fungi. Microbiological literature contains many references (*e.g.,* 46) to the effects of radiation upon respiration and fermentation of various bacteria, yeasts and molds. Only that portion of this literature in which the effects on the metabolic processes can be distinguished, to some degree at least, from the more general effects on growth and reproduction, will be considered here.

One of the earliest reports is that of Schutzenberger and Quinquaud[1] who in 1873 measured the oxygen consumption of beer yeast suspensions. No differences in rate were found in darkness, in diffuse light, or in direct sunlight. No data on temperature were included. More recently, the rate of aerobic oxygen consumption or of anaerobic carbon dioxide production of baker's yeast in glucose solution was found to be substantially the same in darkness and in light of fairly high intensity (75-watt tungsten filament lamp at 4 cm. distance) (314). According to Tang, exposure of *Saccharo-*

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[1] Since every author and article mentioned in the text is cited in the bibliography, reference numbers are given only when necessary to avoid confusion.

myces wanching to the visible portion of the radiation from a quartz mercury arc did not affect the subsequent rate of oxygen consumption in darkness. Similar results were reported by Schneider (263).

Van der Paauw found a 20% increase in oxygen consumption of *Saccharomyces vordermanii* during illumination, presumably by a 150-watt lamp at about 14 centimeters distance, filtered through eight to nine centimeters of water.

Dumas stated that yeast fermentation proceeded more slowly in darkness than in light. According to Lubimenko and Froloff-Bagreief, exposure of raisin must, inoculated with yeast, to daylight over a long period of time resulted in an approximately 20% decrease in the amount of carbon dioxide produced, as compared with the dark control. The decreases in amount of sugar fermented and in amount of alcohol formed in light were somewhat less.

Von Euler and Laurin reported that a 30-minute exposure to sunlight produced a 5% decrease in the fermentation capacity of yeast. Polarized light was stated to stimulate fermentation of sucrose by *Saccharomyces cerevisiae* (182). Guerrini, who measured the fermentation of glucose by yeast, reported a greater rate of carbon dioxide production in light than in darkness (114–116, 118). The order of effectiveness of the light was stated to be red > yellow > green > blue > white, although the intensities do not appear to have been equalized in the various spectral regions.

Irradiation by the wavelength band 5,000–6,500 Å was reported to increase the fermentation rate of yeast (111). The stimulation persisted for some time after the irradiation had been discontinued. Murakami exposed koji-extract cultures of *Saccharomyces cerevisiae* and *S. ellipsoideus* to various wavelength bands of visible plus infrared radiation (of unequalized intensities) during a 96-hour incubation period. Determinations of alcohol, aldehyde, acetal, ester and acids showed small and irregular differences, of which the significance is rather doubtful, especially as only a single experiment was performed (199; see also 198, 200).

Rubenstein, using the Warburg technic, measured oxygen consumption by *Sarcina lutea*. Under starvation conditions at 37° C., the initial respiration rate was as much as five times as great in darkness as in Mazda radiation, filtered through water and glass, of about 556 meter-candles intensity. By the end of 48 to 72 hours the oxygen uptake fell to practically nil; the total quantity of oxygen absorbed was approximately the same in the irradiated and in the dark cultures. A quite different result was found at 20°, at which temperature the rate of oxygen consumption was increased by illumination. At about 30° the respiration rate was the same in light and in darkness. At 37° the rate was decreased by illumination, also in the presence of low concentrations of glucose, but in higher glucose concentrations this decrease was said to be much less marked. The oxygen consumption in darkness could be described by the equation for a monomolecular process, but this was not true of the oxygen uptake in light. If the dependency of the light effect upon temperature and substrate concentration is of general validity, it may perhaps account for many of the discrepancies noted by other workers.

The effects of various spectral regions, obtained by means of filters, on the formation of gas by *Bacillus proteus vulgaris* were singularly consistent for a wide variety of substrates (117). In comparison with the dark control, the relative average gas production was 1.6 in green, 1.2 in yellow, 1.1 in white, 0.9 in red, and 0.6 in blue light. The intensities of the different wave-length bands do not appear to have been equalized.

In 1882, Wilson, in a brief paper, mentioned that light was without appreciable influence upon the carbon dioxide evolution of unspecified mushrooms. Although experimental details were omitted, it was stated that the temperature was controlled. Shortly thereafter, Bonnier and Mangin published the results of a large number of experiments which appear to have been carefully done and fairly well controlled, although the air temperatures were read presumably only to 0.5° (25). Carbon dioxide evolution, oxygen consumption, or both simultaneously, were measured, using both continuous aeration and constant volume methods. Diffuse sunlight was used as the source of illumination, and athough no intensity measurements were made, the effects of high and low intensities were compared in a few cases. In all, there were 18 experiments with the fruiting bodies of *Polyporus versicolor, Agaricus velutipes, A. conchatus, A. campestris* and *Telephora tremelloides,* and with mycelium of *Phycomyces nitens.* Without exception higher rates of respiration were observed in darkness than in light, the difference varying from 2%

to 56% and averaging about 23%. Reduction in respiration was somewhat greater with the illumination of higher intensity. On the average, the light effect was the same whether measured as carbon dioxide evolution or as oxygen consumption, and the respiratory quotient remained substantially the same in light and in dark. In general, the respiration was measured during successive light and dark periods of one to three hours duration. There is no evidence in the data which would indicate that the influence of the illumination conditions carried over into the subsequent dark period.

Bonnier and Mangin compared also the effects of the shorter and the longer portions of the visible spectrum from sunlight; the separation was effected by means of either filters or a prism. No attempt was made to equalize the incident intensities, but if an approximately equal energy separation can be assumed for the prism experiments, it was found that the reduction in respiration occasioned by illumination was due almost entirely to the yellow-red region, the blue-green portion of the spectrum having relatively little influence. The filter experiments, although of less value for the reason mentioned, pointed to the same conclusion.

Purjewicz noted that only in the very young stages and in the mature condition does the respiration rate of mushrooms remain constant over a period of several hours, even under constant environmental conditions, and he suggested that disregard for this situation may have introduced an error in the results of Bonnier and Mangin. The consistency of the data of these workers, however, seems to discount the possibility that such an error could have been of a magnitude sufficient to alter the trend of the results. Purjewicz measured the carbon dioxide production in a series of successive light and dark intervals of 30 to 90 minutes each. Illumination was provided by diffuse sunlight, filtered through a layer of water. The temperature of the respiration vessel was held constant within a few tenths of a degree. Forty-three experiments were performed with the following species: *Agaricus campestris, A. integer, A. melleus, Amanita phalloides, Armillaria mellea, Boletus edulis, Cantharellus cibarius, Lactarius deliciosus, Polyporus versicolor.* With a single exception all the experiments confirmed the findings of Bonnier and Mangin that the respiration rate was lower in light. Omitting the one aberrant case, the ratio light rate/dark rate varied between 0.58 and 0.90, and the average decrease of the respiration rate in light was

22%. The data give no suggestion of an after-effect of one period on the following.

Twelve additional experiments were made by Purjewicz comparing the relative effectiveness of red and blue light obtained by means of filters. It was observed that the reduction of respiration in the red light was approximately equal to that in white light, whereas in blue light the effect was only about one-fourth as great. The quantitative significance of these values is somewhat doubtful, however, in view of the lack of information as to relative intensities of the two spectral regions used.

In the same year Elfving reported the results of an investigation on the carbon dioxide production of several species of molds (*Briaraea* sp., *Aspergillus niger, A. flavescens, Mucor racemosus, Penicillium glaucum*) measured during successive dark and light intervals of one to two hours each. Illumination was provided by direct or diffuse sunlight filtered through water and glass. The temperature was controlled within 0.7°. In 25 experiments with 4- to 19-day-old cultures growing on a variety of organic substrates, there was found no effect of light greater than the experimental error of measurement which was stated to be about 12%.

In further experiments with *Briaraea* and *Penicillium glaucum* designed to test the possibility that a different behavior might be found in younger cultures, Elfving measured the total carbon dioxide liberated during the first few days following inoculation, in duplicate cultures growing in daylight or in darkness. In most cases the carbon dioxide production was considerably greater in the dark, the ratio carbon dioxide produced in light/carbon dioxide produced in dark ranging from 0.37 to 1.0. The smallest differences were found in the media containing peptone. The author concluded that these results were in complete agreement with those of Bonnier and Mangin (25). However, the results of these experiments are complicated by differences in growth, inasmuch as the dry weights of mycelium produced were also greater in darkness than in light. On the basis of carbon dioxide evolved per unit dry weight of mycelium produced, the differences between the illuminated and non-illuminated cultures were relatively small; the average ratio carbon dioxide produced per unit dry weight in light/carbon dioxide produced per unit dry weight in dark for all the experiments equalled 1.10. Considering the variability of replicate experiments, this does not appear to indicate a significant difference between light and dark cultures. The average value of the above ratio for the various culture media without peptone was 1.19, whereas for those containing peptone it was 0.98. The significance of this difference is not clear, but it suggests that the composition of the substrate may play, directly or indirectly, an important rôle in the respiratory response to light.

A few additional experiments with young cultures, similar to the above, were carried out to compare the respiration in blue and in yellow-red light. The total carbon dioxide evolved was greater in the blue light, but on the basis of carbon dioxide produced per unit dry weight, there was no significant difference.

Detmer (1880, p. 271; 1882, vol. 2, p. 133; 1893) repeatedly referred to his experiments on respiration of fungi, reporting without any details his finding that the respiration is the same in light and in darkness. Aereboe reported a few experiments with young fructifications of *Agaricus campestris*. He could observe no effect of direct sunlight (filtered through 30 centimeters of alum solution) on rate of carbon dioxide evolution.

Using a technic essentially similar to that of Purjewicz, Shorawsky studied the influence of light on respiration of *Agaricus campestris, Phycomyces nitens* and *Mucor* sp. The alternate light and dark periods were of 20 to 60 minutes duration. No details as to the age or culture conditions of the material were given. The results with *Agaricus* confirmed those of Bonnier and Mangin (25) and of Purjewicz; in 10 experiments the average ratio carbon dioxide produced in light/carbon dioxide produced in dark was 0.82. A stimulatory effect of light on respiration was observed with *Mucor;* the average ratio for 12 experiments was 1.17. With *Phycomyces* the individual experiments were rather inconsistent: of six experiments two gave an increase, two a decrease, while two showed no significant difference; the average value of the ratio carbon dioxide in light/carbon dioxide in dark was 1.02. Aside from the somewhat erratic results with *Phycomyces* the data contain no suggestion of a carry-over of the effect from one period to the next.

Kolkwitz measured carbon dioxide production by *Aspergillus niger, Penicillium* sp., *Mucor* sp., *Oidium lactis, Micrococcus prodigiosus* and *Proteus vulgaris.* An electric arc with a metal reflector provided radiation intensities up to 60,000 meter-candles. It was stated that the infra-red was filtered out in all cases and the ultra-violet also in some. Temperature was apparently carefully controlled. In most of the experiments the nutrient medium in which the culture had grown was replaced a day or two before the respiration measurement by water, by sugar solution, or by fresh nutrient medium. Since, under some conditions, oxalic acid is decomposed by light with liberation of carbon dioxide, the author discarded all experiments in which oxalic acid was present at the end of the run. Usually the respiration rates were sufficiently great so that several measurements could be made during each light and dark period. Despite a considerable variability of the respiration rate which was frequently found under constant conditions, most of the experiments with *Aspergillus* and *Penicillium* showed an unmistakable increase of respiration in light. This increase, which amounted to 5% to 20%, was usually transient, the respiration curves passing through a maximum at about 15 to 30 minutes after the start of illumination. The results with the other organisms, while not as clear cut, also indicated a greater respiration in light than in dark. Kolkwitz stated that the light effect was the same whether high or low intensities were used. An experiment with *Aspergillus,* in which the blue end of the spectrum was filtered out, also gave a stimulation.

More or less similar experiments were performed by Maximov, who attempted to reduce the irregularities in the dark respiration rate by more adequate provision of nutrient. Using *Aspergillus niger,* he found that the influence of light was related to the age of the culture and to the nutrient conditions. With an abundant nutrient supply the respiration of young cultures was not affected by illumination (of unspecified intensity) furnished by an electric arc lamp; exposure to direct sunlight, however, did result in an increased rate. Even with the artificial source a clear-cut stimulation was observed with young cultures deprived of nutrients and in old cultures regardless of the nutrient supply. The increase in respiration rate, which amounted to 10% to 40%, was manifested in the first measurement after illumination (usually 30 minutes) and thereafter became progressively smaller. With a long series of alternate light and dark periods repeated stimulations could be observed. Light was found to increase the respiration also of *Mucor stolonifer* during the first 30 minutes, but subsequently the mold appeared to be injured.

An improvement in technique was introduced by Löwschin who measured the temperature of the respiring material itself, as well as that of the thermostat. The species studied were *Aspergillus niger, Cladosporium herbarum, Oidium lactis* and *Penicillium* sp. Illumination was furnished by diffuse daylight filtered through water and glass. In all, 22 experiments were performed from which the writer concluded that no regular increase of respiration unconnected with heating of the culture was ever found. Examination of the data presented reveals, however, that the results were rather inconsistent. Thus, in one experiment during a 30-minute light period, the respiration rate increased 10% while the temperature rose 0.5°; in the 30-minute dark period immediately following, the respiration increased by an additional 5%, although the temperature decreased 0.2°. Löwschin did not determine the temperature coefficient for the dark respiration of the material used, so that one can not be certain that the temperature increases noted could indeed explain the observed respiration increases. Furthermore, in at least two experiments illumination was accompanied by a decrease in respiration.

Richards (250) measured carbon dioxide production, and in some cases also oxygen absorption, by young sporophores of *Marasmius conigenus, Polyporus adustus, Coprinus comatus, C. micaceus, Hypholoma fasciculare, Lactarius quietus* and *Polystictus versicolor*. Experimental data were not given, but it was stated that no significant difference in respiration rate was found in dark and in weak diffuse daylight or electric light of moderate intensity. On the average, the respiratory quotients were also identical in light and in dark.

De Boer, working with mycelia of *Phycomyces blakesleeanus* and *Polyporus destructor* and small fructifications of *Lactarius rufus* and *Laccaria amethysta,* was unable to detect any effect on respiration by diffuse daylight or electric light at intensities of 800 or 6,000 meter-candles. According to Pasinetti and Grancini, 25- to 50-minute exposure of *Alternaria brassicae* to direct sunlight resulted in a diminution of the subsequent rate of gaseous exchange.

In disks of young fructifications of *Psalliota campestris,* Föckler observed a 27% increase of oxygen uptake during the first hour of exposure to sunlight; in the next hour of illumination the respiration remained nearly at this higher level and then on subsequent darkening fell to the original dark value. There was little or no after-effect of the light treatment. A similar rise in respiration was produced also by incandescent lamp radiation from which the infra-red and long visible red had been filtered out. Others stated that the rate of oxygen consumption by molds and bacteria was not influenced by exposure to red or blue light (of unspecified intensity) (197).

Considering the work on fungi as a whole, the discordant findings of the various investigators are very striking. Of the experiments using mushroom sporophores, the most extended and consistent data are in rather good agreement, showing a decrease of about 20% in light (25, 241, 272). Of the investigators who reported no effect of light on respiration, some do not present the experimental data (68, 69a, 69b, 250, 323), while others performed only a limited number of experiments (3, 64). The sole claim of an increased respiration in light was apparently on the basis of only two experiments (88).

The results with molds are even less consistent and give evidence that the condition of the material, as well as the nature of the substrate, may profoundly modify the influence exercised by light. None of the investigators seems to have attempted to control the illumination conditions prior to the respiration measurements, a factor which, conceivably, may play an important rôle, especially in view of the marked influence of light on development. The factor of culture age which appears to be of considerable significance may possibly be related to the development and maturation of spores which would tend to affect the light-absorbing properties of the culture. It should be mentioned, however, that the presence or absence of spores seemed to make little difference in the light stimulation in some of Maximov's experiments, while de Boer found that in cultures of *Phycomyces* on bread the sporangiophores were responsible for only 10% to 15% of the total respiration.

Of the indirect explanations which have sometimes been advanced to account for the observed light effects, temperature increases on illumination could scarcely explain the cases of decreased respiration. Furthermore, Kolkwitz obtained similar stimulations at 27.5° and 41°, temperatures at which the temperature coefficients might be expected to differ greatly. A purely photochemical decomposition of organic acids, such as is well known to occur *in vitro* (*e.g.*, 71, 78, 235), could perhaps explain some of the stimulations observed; again, this would not be true of those experiments which showed a decreased respiration in light. None of the work with molds has included measurement of the respiratory quotient. Such measurement would appear to offer a possible means of detecting photoxidations of organic acids, since the ratio of carbon dioxide produced/oxygen consumed is greater than unity for such reactions.

While the experimental methods have scarcely been adequate to yield an unequivocal result, it may be stated that there is no clear-cut evidence in any of the work of an after-effect of a light treatment on the subsequent dark respiration. In the experiments in which the rôle of light quality has been studied, the long wavelength end of the spectrum has usually been claimed to be the effective portion, whether in inducing a stimulation (154) or an inhibition (25, 241) of the respiration.

The majority of workers who have studied the influence of visible radiation on yeasts have found the respiration rate unaffected; the sole exception is van der Paauw. Stimulation of the fermentation rate, on the other hand, has been reported by several, though not all, investigators. From the three studies dealing with bacteria it has been concluded, respectively, that the respiration is stimulated, inhibited and not influenced by light.

This cataloging of the results of previous investigators is not intended to carry the implication that such a procedure is capable of demonstrating the truth or falsity of any given report. The conclusion to be drawn from the complete lack of agreement would appear to be that each particular type of material must be subjected to much more intensive study with proper regard for the many factors which may influence its reactions. Indeed, because of the variety of experimental subjects, conditions and technics which have been employed in the several investigations, comparisons among them may not be justified at all.

Normally chlorophyll-deficient organs and tissues of higher plants. a. Flowers and Fruits. Cahours reported the results of experiments on the respiration of flowers but gave no experimental details (51). According to Aereboe, *Ranunculus* spp., Liliaceae, *Nymphaea alba, etc.,* were used. The carbon dioxide production was stated to be, in general, slightly greater in light than in dark, the difference being much more marked in pure oxygen than in air. Flowers of *Salvia* *pratensis* freed of all green parts were found to produce 4% to 20% more carbon dioxide in diffuse light than in darkness, the temperature of the respiration vessel remaining constant (69). No such increase was observed with flowers of *Syringa vulgaris* or with petals of *Rosa,* which the author considered as additional evidence that the positive results with *Salvia* were not due to a heating effect.

Using methods similar to those described above in the section on fungi, Bonnier and Mangin (26) studied the respiration of inflorescences of *Arum maculatum, Hyacinthus orientalis* and *Robinia pseudo-acacia.* The *Arum* inflorescences were stated to be free of chlorophyll. In the experiments with hyacinth and locust the bases of the inflorescences were inserted in moist soil in the respiration chamber; after the respiration was determined the flowers were picked off and the measurements repeated with the peduncles alone, using air containing carbon dioxide. Bonnier and Mangin published only the results after correction for the photosynthetic activity of the green parts. Their data show a 14% to 34% reduction of respiration during the period of exposure to daylight. The respiratory quotient was the same in light and in dark. Small temperature changes would not appear to be of importance in this work, since in the experiment with *Robinia* a difference of 3° did not produce any alteration of the dark respiration rate.

The respiration rates of allegedly chlorophyll-free flowers of *Lilium candidum* and *Nymphaea alba,* as well as of chlorophyll-containing inflorescences of *Lathraea squamaria,* were measured by Purjewicz. Although the temperature was controlled within quite narrow limits, the results were so irregular that they seem of little evidential value.

Aereboe carried out an extensive series of experiments with petals of *Taraxacum officinale, Syringa vulgaris, Paeonia, Salvia pratensis, Crepis biennis, Chrysanthemum leucanthemum, Papaver rhoeas,* garden aster, *Rosa centifolia* and some other roses. All the material was shown by spectroscopic examination to be free of chlorophyll. The temperature was measured by a thermometer in the respiration vessel with its bulb surrounded by the respiring material. Interpretation of several of the experiments is rendered difficult by a continuous fall in the respiration rate with time. In those instances where this was not the case, the difference between respiration in dark and in diffuse or direct sunlight fell within the range of experimental error. It has been pointed out by de Boer, however, that there may be some question as to whether the petals were not packed so closely in Aereboe's experiments that only a small fraction of the material was actually exposed to the light.

Chlorophyll was presumably present in most of the flowers studied by Curtel, so that the larger part of his data does not appear to be of significance for the problem here considered. In *Phlox paniculata,* of which it was stated that the green parts were restricted to the very small calyx, both the carbon dioxide production and the oxygen consumption were appreciably less in diffuse sunlight than in darkness. Montfort and Föckler stated, without further details, that the ratio of respiration in sunlight to that in darkness for the chlorophyll-poor inflorescences of *Neottia nidus avis* was 1.57.

Ranjan and Saksena measured the rate of carbon dioxide production of flowers of *Canna, Nerium* and *Bougainvillea* before, during and after an 8- to 12-hour period of exposure to the radiation, presumably filtered through water and glass, of a 1,500 watt lamp at about one foot distance. In *Canna* only a slight indication of a respiration increase was found. The carbon dioxide evolution of the *Nerium* flowers, on the other hand, rose after a few hours of exposure to about one and one-half times that of the initial dark rate and remained nearly at this level during the rest of the illumination period. In the subsequent dark period the respiratory stimulation persisted for many hours in the pink-colored flowers, whereas yellow flowers exhibited only a small and transient after-effect. The flowers of *Bougainvillea* showed an increase of about 40% during the first two hours of illumination, but on continued exposure the rate returned nearly to the previous dark value. From some rather crude estimates of the amounts of carotenoids and anthocyanins present in the various flowers, the authors concluded that the light effects were correlated with the pigment concentration.

Cahours, who measured the oxygen absorption and carbon dioxide excretion by ripe apple, orange and lemon fruits, stated that the proportion of carbon dioxide produced was considerably greater in diffuse light than in darkness (50).

b. Seeds and Young Seedlings.[2] As a matter of historical interest mention should be made of the experiments of Pauchon, although they do not appear very decisive for the problem under consideration. This worker measured the gaseous exchange of several species of seeds which were permitted to germinate in light or in darkness for a period of 4 to 11 days. In 10 of 12 experiments the oxygen consumption was 20% to 95% greater in the light. Carbon dioxide production was more nearly constant under the two conditions; as a result the respiratory quotient was always greater in darkness. In these experiments no attempt was made to control the temperature, and in some cases the degree of germination and development differed in the light and dark sets; these circumstances may perhaps suffice to explain the observed results.

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[2] In this section are included references to young seedlings which might be expected to exhibit little or no photosynthetic activity, though germinated in light. Studies of older seedlings cultured in darkness, so as to prevent chlorophyll formation, are discussed below in the section on etiolated plants.

Bonnier and Mangin (26) measured both carbon dioxide production and oxygen absorption by young seedlings of *Lepidium sativum, Linum usitatissimum, Lupinus luteus* and *Faba vulgaris* during alternating light and dark periods of one to two hours. In all of the 13 reported experiments the respiration was higher in darkness than in daylight by an amount up to 46%, the average difference being about 15%. The magnitude of the effect was said to vary directly with the light intensity. The authors stated that the retarding influence of light on respiration was smaller during the early stages of germination, a result which they regarded as being due, in part at least, to the opacity of the seed coats; it was found, indeed, that the light effect was less in seeds with opaque coats than in those with transparent integuments. The carbon dioxide/oxygen ratios found by Bonnier and Mangin were quite low, ranging from 0.30 to 0.87. Since the photosynthetic quotient may be assumed to be unity, the occurrence of an appreciable amount of photosynthesis would be expected to result in a lower carbon dioxide/oxygen ratio in light than in darkness. As there was no consistent indication of such a difference, it may be concluded that, notwithstanding the presence of chlorophyll in some of the seedlings used, photosynthetic activity was not sufficiently great to account for the observed light effects.

Respiration of barley and wheat germinating in diffuse daylight and in darkness was measured by Day, presumably with a high degree of accuracy. Although the light-grown seedlings were apparently capable of some photosynthesis, the author concluded, from experiments in which the respired carbon dioxide was not allowed to accumulate, that light had a small stimulatory effect on the respiration, amounting to about 4% or 5%, whether measured in terms of carbon dioxide or of oxygen.

Becquerel measured the gaseous exchange which had occurred during a five-month period of storage in light or in darkness of various dormant seeds, seed coats and seeds with the integuments removed. Since the respiratory quotients, in general, departed quite widely from unity, there must have occurred a change in volume or pressure during the experiment. It is not clear from the published data that correction was made for this change. Interpretation of the data is handicapped also by a number of misprints or other errors. Despite these uncertainties the general result is very clear: in every case the illuminated material had respired to a greater extent than the dark material. The difference varied from 20% to 570%. The same general result was found in both carbon dioxide production and oxygen consumption, although quantitatively the effect was in some cases quite different by the two methods; in other words, the respiratory quotients differed in light and in dark. The significance to be attached to Becquerel's findings is rather doubtful in view of *a*) the lack of any mention of temperature control, *b*) the total absence of respiration in four of the dark experiments, and *c*) the curious finding that in castor beans, peas and beans the respiration of the integuments alone was as great as or greater than that of the intact seeds or of the seeds minus the coats.

In respiration studies of seeds of *Cucurbita pepo,* with seed coats removed, irradiation by moderately high intensity electric light, with the infra-red filtered out, produced a 20% increase during the first hour (88). In sunlight a 56% increase was noted; during the second hour of illumination the rate was only about 35% higher than in the initial dark period, and on subsequent darkening it fell to the original dark value. Sunlight from which the ultra-violet was removed by ordinary glass caused only about one-half the effect found when a Uviol filter, transmitting the longer ultra-violet, was used.

Quite different results were found with isolated cotyledons of *Cucurbita pepo* (45). One cotyledon was exposed continuously to light of about 100 foot candles intensity; the other cotyledon from the same seed was kept in darkness. Measurements of the gas exchange were made at intervals from the 18th to the 48th hour after excision. In the darkened organ the respiratory quotient remained at about 0.6 throughout this period. In light the initial rate of oxygen consumption was only one-third that in darkness, while the rate of carbon dioxide production was about one-half; the respiratory quotient was 0.9. Thereafter the rate of gaseous exchange of the illuminated cotyledon approached that in darkness and the respiratory quotient gradually fell to 0.7. Brown suggested that light retarded the conversion of fat to carbohydrate.

A rather special case is furnished by seeds whose germination is influenced by light, the so-called light-sensitive seeds. With tobacco seeds which require a light exposure to initiate germination, a relatively short irradiation period resulted in an immediate and persistent increase in respiration rate (measured either as carbon dioxide evolution or as oxygen absorption) over that of the unilluminated controls (152, 265). Schröppel found, further, that appreciable increases in the catalase and peroxidase activities of the seeds did not occur until several hours after the light treatment.

c. Roots. In a single experiment on the oxygen consumption of excised roots of *Vicia faba* during alternate light and dark periods, no significant differences were observed (310). Chlorophyll-free roots of *Phaseolus multiflorus, Primula officinalis, Sedum maximum, Vicia faba* and *Zea mays* were also investigated (241). The successive respiration determinations showed marked irregularities. These were in turn reflected in the results which often were inconsistent even for a single species. Aereboe performed three experiments with excised *Vicia faba* roots. The differences found appear to be within the limits of experimental error.

Montfort and Föckler, and Föckler described experiments concerning the influence of light on oxygen uptake of excised roots of *Hyacinthus candicans* and *Vicia faba.* Illumination by electric light minus the infra-red, or by sunlight, gave increases of 15% to 100% over the dark rate. At the higher intensities used, the maximum increase occurred during the first hour of irradiation; on continued exposure the respiration fell to a more or less constant value below this maximum but still above the dark level. On subsequent darkening the respiration returned nearly or quite to the original dark value, and a second stimulation similar to the first could be evoked by further illumination. In the *Vicia faba* experiment, which was stated to have been done in sunlight of lower intensity, the first increase on illumination was maintained during a three-hour exposure period.

The effect of intensity was studied with the incandescent lamp source. The stimulation was roughly proportional to intensity over the range covered (stated to be approximately from 4.5% to 20% of the intensity of the summer noonday sun).

The rôle of light quality was studied by means of various filter combinations which furnished equal energy values in the regions: 600 to ca. 700 mμ, 470 to 620 mμ and ca. 400 to 500 mμ. The stimulations found were: red, 7%; green, 10%; blue, 37%. The spectral effectiveness curve agrees fairly well with the absorption curve for the etiolated leaf, as determined by Seybold. The infra-red region appears to be without effect, since nearly identical results were obtained with electric light whether the infra-red were filtered out or not. The influence of the ultra-violet was examined by comparing sunlight transmitted through ordinary glass and through Uviol glass; white light alone caused a 22% stimulation, whereas the white light plus ultra-violet produced a 33% increase.

It was also found by Föckler that roots which had been growing exposed to light for some days prior to the respiration measurements showed a much smaller initial stimulation on illumination, from which he concluded that the roots can become light-adapted.

Mothes, Baatz and Sagromsky stated, without details, that the rate of oxygen consumption of roots was not influenced by exposure to red or blue light.

Using the Fenn microrespirometer, with excellent temperature control, Marsh and Goddard made some incidental measurements of the oxygen uptake by carrot root slices in darkness and in light. The radiation was provided by a 100-watt Mazda lamp at 10 centimeters distance. The results of only a single experiment are shown in detail, the readings being taken at 20-minute intervals. During an hour's illumination the respiration rate was fairly constant but only about 75% as great as that in the preceding dark period. A further reduction to approximately 60% of the initial dark value was found during an hour of darkness following the exposure. In the three additional experiments for which the results are reported, the ratios of light respiration/dark respiration were 0.88, 1.02, and 1.40.

d. Tubers. The oxygen consumption of disks of potato tubers was found to increase by 21% during the first hour of illumination by incandescent electric light, minus the infra-red, at an intensity approximately one-fifth of noon sunlight; exposure to full sunlight resulted in a 51% rise over the dark rate. During the second hour of illumination the respiration fell nearly to the dark value (88).

e. Rhizomes. Bonnier and Mangin (26) determined gaseous exchange by rhizomes and adventitious roots of *Solidago virgaaurea* and *Epilobium spicatum* in darkness and in sunlight. In light the carbon dioxide production was 10% to 34% less than in darkness, while the oxygen consumption was decreased by only 4% to 16%. As a consequence, the respiratory quotient was, in all cases, somewhat smaller in light. No significant effect of light on the carbon dioxide evolution by rhizomes of *Polygonatum multiflorum* could be observed by Purjewicz in a limited number of experiments.

f. Buds. Bonnier and Mangin (26) found no difference in the carbon dioxide production by unopened buds of *Aesculus hippocastanum* in light and in dark. The authors suggested that the lack of effect may have been due to the opacity of the bud scales. Johansson's (141) measurements of carbon dioxide production by young fronds of *Polypodium vulgare,* prior to development of the photosynthetic capacity, indicated that light does not influence the respiration.

Surveying the work on flowers, seeds, roots, tubers, rhizomes and buds as a whole, it seems fair to say that, while very few of the investigations are free from methodological objections of one kind or another, there are definite indications that radiation influences the rate of respiration in some materials. For the most part increased gas exchange has been observed as the result of irradiation, although in a few instances the opposite effect has been found. The experiments thus far reported are to be regarded as exploratory in nature. In no case have there been carried out systematic studies employing adequate experimental technics with sufficient attention to the biological factors involved.

Saprophytic, parasitic, albinic and variegated angiosperms. Drude measured the carbon dioxide production of entire flowering plants of *Monotropa hypopitys* at one- to five-hour intervals over a three-day period, during which they were exposed to diffuse sunlight during the day. From several experiments, for which the data were not published, the author concluded that the illumination had no consistent influence on the respiration rate, although in a number of cases the carbon dioxide evolution was greater during the day. It does not seem possible to determine, from the recorded data, the extent of the temperature fluctuation of the respiring material.

Wilson, in a preliminary account which apparently was never followed by a more extensive publication, stated only that in numerous experiments with *Orobanche, Monotropa* and *Hypopitys* there was no effect of light on carbon dioxide production.

In the experiments of Bonnier and Mangin (26), plants of *Monotropa hypopitys, Orobanche epithymum* and *Neottia nidus-avis,* together with considerable amounts of soil and humus, were used. As the substrate was not illuminated its gaseous exchange was assumed to remain constant. In light the respiration was decreased by 13% to 44%; quite similar results were found for both the carbon dioxide production and the oxygen consumption.

Groner compared the carbon dioxide production by albino corn seedlings in darkness and when exposed to Mazda illumination of moderately high intensity filtered through water. No differences outside the experimental variability (which was probably of the order of several per cent) were found when the plants were illuminated continuously or kept in darkness during a three-and-one-half-day period. In two experiments, in which the irradiation followed a period of darkness, respiratory increases of approximately 50% and 100% were observed.

In one of these the maximal stimulation occurred during the first two-hour period of illumination; by the end of 21 hours in light (and possibly much earlier) the rate had returned to the initial dark value. The other experiment showed a slight diminution of respiration during the first two hours of exposure, the maximal increase occurring in the succeeding two-hour interval; in this case, too, the stimulation was only transient. There seems to be a suggestion in the published curves that a slight increase in respiration occurred also when the plants were darkened after a light exposure.

Van der Paauw found an increase of the order of 35% in the oxygen consumption by detached leaves of *Oplismenus* during irradiation. The radiation was presumably furnished by a 150-

watt lamp at about 13 centimeters distance, filtered through eight to nine centimeters of water and applied for an hour. Following exposure the respiration returned to the original dark value. Ranjan (242, 244, I) reported the results of experiments with detached chlorophyll-poor leaves of *Croton*. During exposure to the radiation, filtered through water and glass, of a 1,000-watt lamp at about one foot distance, the rate of carbon dioxide evolution was approximately 25% greater than that in the preceding and subsequent dark periods.

Parija and Saran measured the carbon dioxide production by detached albinic and variegated leaves of *Aralia* before and after illumination. The radiation was produced by a 60-watt Argenta lamp at 10 inches distance and was filtered through a layer of water. Various spectral regions were isolated by means of Wratten filters. Although it is stated that the intensity was the same in all cases, it is not clear that this refers to the light incident on the leaf. Illumination was without influence upon the respiration rate unless the detached leaves had been kept previously in darkness for two to three days. In such starved leaves respiratory increases, presumably as great as 150%, were elicited by irradiation. Exposure for seven and one-half minutes was as effective as that for two hours. The greatest increase was produced by violet light; blue and white light were considerably less effective, while red light was inactive. Parija and Saran found also that the reducing sugar content of the starved leaves was greatly increased by a seven-and-one-half-minute exposure. Based upon the sugar content of the leaf prior to illumination, the increase varied from 100% to 700% in several experiments; the absolute gains per unit weight of leaf were quite uniform, however.

The conclusions expressed at the end of the preceding section would appear to apply equally well to the experiments with albino plants, saprophytes and parasites.

Etiolated plants. The earliest measurements of the influence of light on respiration which have come to the reviewer's attention are those of Morot (1849), made incidental to a study of chlorophyll formation. Excised etiolated oat leaves were found to produce 0.84 cc. of carbon dioxide in darkness, and 1.2 cc. in direct sunlight, in which case they remained yellow; in diffuse sunlight 0.82 cc. of carbon dioxide was evolved, the leaves becoming green. The temperature was not controlled or measured.

Von Wolkoff and Mayer reported results typical of a large number of experiments on oxygen consumption by etiolated seedlings of wheat, nasturtium and buckwheat; in some experiments the cotyledons and/or leaves were removed. The temperature fluctuated rather widely and the analytical error was relatively large with respect to the magnitude of the respiration rates. Exposure to sunlight, or to sunlight with the red region filtered out, resulted in some cases in small but consistent increases in respiration, whereas in others no marked differences were found. The authors expressed uncertainty as to whether the observed effects were to be ascribed to a real stimulation which occurred only under certain conditions or to limitations of the technic employed. Wilson observed no effect of light on the respiration of etiolated seedlings of various species.

In a limited number of experiments with etiolated seedlings of *Ricinus communis, Lepidium sativum, Triticum sativum* and *Linum usitatissimum,* Bonnier and Mangin (26) consistently obtained a smaller respiration in light. The decrease amounted to 11% to 26% when measured as carbon dioxide evolution; in two experiments in which oxygen absorption was determined simultaneously the decrease was only 4% and 6%. Purjewicz measured carbon dioxide production by etiolated seedlings of *Zea mays* and *Lepidium sativum* at low temperatures (4° to 6°) in order to prevent chlorophyll formation during the illumination. Although the data for the five individual experiments are somewhat irregular, all showed, on the average, a greater respiration in light by 4% to 22%.

Yellow chlorophyll-poor cells of *Chlorella* incapable of photosynthesis were obtained by culturing in a sugar-containing medium low in iron (79). From measurements of the gas exchange, it was concluded that the respiration of such cells is the same in light as in darkness. There may be some question as to the validity of this conclusion, however, since the method employed depends upon a knowledge of the value of the respiratory quotient, which was not determined. It has been shown by others that the R.Q. of many green algae in the presence of sugar is not unity, and it may possibly differ in light and in darkness.

Föckler observed a 31% increase in absorption of oxygen during the first hour of exposure to sunlight of disks of young etiolated shoots of *Asparagus officinalis.* During the second, third and fourth hours of illumination the increases over the dark rate were approximately 20%, 19% and 12%, respectively. In the following dark period the rate returned to the original dark value. Illumination by electric light minus the infra-red, at about one-fifth full sunlight intensity, resulted in only a 7% rise in respiration.

The influence of white light on carbon dioxide production by etiolated barley seedlings has been studied, using the spectrographic method of analysis (143, 316a). On exposure to light of fairly low intensity (180 foot-candles or less), no significant alteration of the rate of carbon dioxide excretion was apparent during the first half hour. Following this, the plants, whether continued in light or darkened, exhibited a rate of respiration which increased to a maximum only after some hours, and then gradually decreased. By 24 hours after a half-hour period of illumination, the dark respiration was substantially the same as that before exposure. At this time a second exposure to light again resulted in an increased rate of carbon dioxide production similar to that previously observed.

The magnitude of the respiratory stimulation was dependent upon the intensity and duration of the irradiation. At an intensity of 60 foot-candles, exposures of 5 or 10 minutes elicited only insignificant effects, whereas illumination for 20 minutes or longer resulted in marked increases. With an exposure time of 30 minutes, the magnitude of the increase in respiration rate, which was relatively independent of the intensity in the range 50 to 500 foot-candles, was approximately 20%. Smaller stimulations were evoked by either higher or lower intensities.

For the most part, the data so far available are insufficient to permit definite conclusions as to the influence of radiation upon etiolated plants. The results of Weintraub and Johnston, although of a preliminary nature, indicate that light induces an appreciable augmentation of the rate of carbon dioxide production by the etiolated barley seedling.

Chlorophyllous Plants

There exists a considerable body of data on the gaseous exchange of chlorophyllous plants which bears more or less directly on the problem of the influence of radiation on respiration. The work containing direct measurements of respiration under conditions in which photosynthesis appears to have been nearly or quite suppressed by the experimental treatment will be considered first, and in a subsequent section there will be indicated some lines of evidence obtained with plants capable of assimilation. This division is to a certain extent merely an expression of interpretation, since it is sometimes impossible to determine whether or not photosynthesis is entirely absent.

Direct evidence under conditions in which photosynthesis is suppressed. It should be emphasized that since there is no certainty, in any of the experiments reviewed in this section, that photosynthesis is entirely absent, only those results which show a greater apparent respiration in light than in darkness can be regarded as significant. Furthermore, the possibility should not be ignored that the methods employed to inhibit photosynthesis may themselves exert an influence on the respiration response to irradiation.

In many investigations of the diurnal course of assimilation under "natural" conditions, there have been demonstrated minima in the apparent rate of photosynthesis during the period of highest light intensity (and of highest temperature). In some instances actual excretion of carbon dioxide at a rate greater than that of the normal dark respiration has been observed (*e.g.*, 157–159, 300). Marked fluctuations have been found also in the diurnal course of the respiration of plants kept in darkness (*e.g.*, 160). Since in such experiments many environmental factors are not controlled, the significance of these results is by no means clear.

a. Suppression of Photosynthesis by Chemical Agents. Bernard appears to have been the first to demonstrate a differential inhibition of photosynthesis and of respiration by chemical agents. This author found that treatment of *Potamogeton* and *Spirogyra* with chloroform caused cessation of oxygen evolution in light while carbon dioxide evolution continued. The photosynthetic capacity was restored on removal of the chloroform. No quantitative comparison of the rates of respiration of the treated plants in light and in dark was made, however. During the ensuing quarter century a number of investigators (for references see, *e.g.*, 150) studied the influence of various narcotics on the gaseous exchange of plants. This literature need not be reviewed here, since the methods employed were not adequate to yield quantitative information concerning the effect of light on respiration.

Measurements of carbon dioxide production by excised shoots of young barley plants and of detached *Prunus laurocerasus* leaves in air containing chloroform vapor were made by Irving. A transient stimulation of respiration due to the chloroform itself was observed, but the rate was practically the same in light as in dark. Warburg (313, I) found that the addition of a small amount of *n*-dodecyl alcohol to an illuminated suspension of *Chlorella* in an alkaline carbonate-bicarbonate buffer irreversibly abolished the photosynthetic capacity and resulted in a greater oxygen consumption than in darkness. Complete inhibition of photosynthesis by a number of other substances was also reported (313, II), but in these instances the rate of oxygen consumption was stated to have been the same in light as in darkness.

Fromageot observed that the photosynthesis of *Ulva lactuca* was first very markedly retarded and finally completely suppressed in the presence of increasing concentrations of glycerine. The dark respiration was also decreased but to a much smaller extent. Although the temperature and illumination conditions do not appear to have been very well controlled, there seems to be no doubt that at glycerine concentrations above about 40% a much higher rate of oxygen consumption occurred in light than in darkness.

Shibata and Yakushiji, in a preliminary note, reported that small amounts of hydroxylamine hydrochloride completely inhibited photosynthesis in *Chlorella ellipsoidea* and *Ulva conglobata* without influencing respiration. The curve shown for *Chlorella* indicates that in the hydroxylamine-treated culture the oxygen uptake is identical in light and in darkness. The results seem much less clear-cut, however, in the more extensive data presented by Yakushiji. Thus, in *Ulva* the conclusiveness of the demonstration that hydroxylamine is without influence on respiration is somewhat vitiated by the inconstant respiration rate, even in the untreated control. Assuming that such is the case, however, the *Chlorella* data indicate a considerably higher respiration rate in light than in darkness, whereas in *Ulva* the reverse is the case. The measurements were made with the Barcroft differential manometer, but only one experiment with each species is mentioned. Although the results do not seem very decisive, the work suggests that hydroxylamine may prove to be a very useful substance for experiments of this kind and worthy of further investigation.

In a quite similar experiment with shoots of *Fontinalis antipyretica,* the rate of oxygen absorption in the presence of 0.001 M hydroxylamine hydrochloride was decreased by only about 2% in darkness, whereas a reduction of approximately 13% was noted in light (302). As has been pointed out above, these findings of decreased apparent respiration in light may indicate merely an incomplete inhibition of photosynthesis.

Föckler measured the oxygen uptake by detached leaves of *Potamogeton lucens* in a 0.06% phenylurethane solution. The respiration rate of the leaves exposed to full summer noon sunlight rose by the end of four hours to more than twice that of the unilluminated leaves; on further exposure the rate diminished somewhat and, on darkening, it returned approximately to the level of the dark control. In comparable experiments with red (600 to 700 mμ), green (470 to 620 mμ), blue (400 to 500 mμ) and white light at about one-fifth of full solar intensity, the oxygen consumption was less than that of the dark control, the differences being most pronounced in the red and green light. Föckler attempted to explain these results by assuming that photosynthesis was not completely inhibited by the phenylurethane and hence tended to mask the respiratory stimulation. On this basis it was concluded that such a stimulation was produced by the white and blue radiation but not by the green or red.

Myers and Burr determined the oxygen consumption by *Chlorella vulgaris* in the presence of 0.01 M KCN over a wide range of measured light intensities. Between 1,500 and 22,000 foot-candles the net oxygen uptake increased with the intensity. Interpretation of experiments in which cyanide is used as a differential inhibitor of photosynthesis is somewhat complicated, both by the relatively large stimulation of the dark respiration induced by the substance itself and by the fact that, under certain conditions at least, the photosynthesis is only partially suppressed. For these reasons it is difficult to determine the lower limit of light intensity which influences the respiration, but it is clear that at intensities above about 13,000 foot-candles the respiration rate exceeded that in the dark; at 22,000 foot-candles the increase amounted to at least 40%.

b. Suppression of Photosynthesis by High Temperature. It has been observed that *Elodea canadensis* placed in water at 45° to 50° completely lost its photosynthetic capacity without any change in its ability to absorb oxygen (266). The authors stated, without furnishing any experimental data, that in such plants oxygen absorption and carbon dioxide liberation continued at the same rate in light as in darkness.

Kreusler measured the carbon dioxide exchange, at various temperatures, of detached leaves and shoots of *Ricinus* and *Prunus laurocerasus*. At 50° C., a temperature which produced signs of injury in the leaves, the carbon dioxide evolution during a two-hour interval, comprising one hour of electric arc illumination and one hour of darkness, was about one and one-half to two times as great as the rate in the subsequent dark period.

Jumelle studied the gaseous exchange, in daylight and in darkness, of several species of lichens, after exposure to elevated temperatures. Although the validity of the analytical method used has been questioned (185), and despite many irregularities in the data which appear to be due largely to individual variations among the plants, certain conclusions seem justified. Exposures of a few hours to several days at temperatures of 40° to 55° resulted in more or less complete suppression of photosynthesis; the extent of the inhibition depended upon the temperature and duration of treatment and upon the species. Following the less drastic treatments there was little or no depression, or even an augmentation, of the dark respiration. After longer exposures and higher temperatures the dark respiration was usually reduced, whereas the respiration in light was frequently increased, in some cases to several times that in darkness. In some experiments oxygen consumption and carbon dioxide evolution were affected to the same degree, but in others the respiratory changes were accompanied by marked alterations of the respiratory quotient.

Wurmser and Jacquot observed that after short exposures to elevated temperatures the photosynthesis (measured at approximately 16°) of *Ulva lactuca* was very markedly depressed, whereas inhibition of the dark respiration was much less pronounced. With two-minute treatments at temperatures above approximately 45° the rate of apparent respiration in light was two to three times as great as the dark respiration of similarly treated plants, although it did not attain the level of the respiration of the untreated dark controls.

Lichens apparently furnish especially suitable material for experiments of this type, since they are able to endure much higher temperatures than many other plants, while the photosynthetic mechanism seems to be quite sensitive to elevated temperatures (see also 282, 283). It has been reported that marked reduction of photosynthetic capacity without change in dark respiration is exhibited also by *Chlorella*, following exposure to elevated temperature (151). A carefully controlled investigation of such organisms, employing modern analytical technics, would appear to offer considerable promise.

c. Suppression of Photosynthesis by Desiccation. Stocker observed that the rate of carbon dioxide production by thalli of *Lobaria pulmonaria*, collected in the air-dry condition, was only about 40% as great in darkness as when exposed to diffuse daylight of about one-fourth to one-third of full sunlight intensity. From Stocker's measurements of the temperature coefficient of the dark respiration, a temperature rise of approximately 8° would be required to account for the observed stimulation on the basis of a heating effect, which does not seem likely under the conditions of the experiment.

d. Suppression of Photosynthesis by Lack of Carbon Dioxide. Photosynthesis can be retarded by removal of the respired carbon dioxide as it is formed. However, since much of this carbon dioxide is produced at or quite near the chloroplasts, it is not possible, in practice, to remove all of it before a portion becomes available for assimilation. Hence the photosynthesis cannot be reduced to zero, and the apparent respiration, as measured by the oxygen consumption, will always be somewhat smaller than the true respiration. Any observed increase in respiration rate under such conditions represents, therefore, a minimal effect. Obviously, only increases in respiration can be detected with certainty by this method.

Measurements of the apparent respiration, in light and in dark, of several species of green plants in the absence of carbon dioxide have been made (26). Unfortunately, the published data are too incomplete to permit of any judgment of the results. In other experiments the rate of carbon dioxide removal appears to have been so slow that considerable assimilation was permitted (191).

Very clear-cut experiments have been made by using the manometric method with potassium hydroxide in the respiration vessel (303). Similar results were obtained with *Oocystis* sp. and with *Hormidium flaccidum*. During illumination (presumably by a 150-watt lamp at about 14 centimeters distance and filtered through 8 or 9 centimeters of water) the oxygen consumption was in some cases more than twice that in the preceding dark period. On subsequent darkening the rate decreased, rapidly at first and then more slowly, returning to the initial dark level after about two hours. Repetition of the exposure at this time resulted in a second stimulation of the same magnitude as the first. An experiment in which the irradiation intensity was reduced to one-fourth gave approximately one-fourth the stimulation. Radiation longer than 600 mμ was apparently much less effective than the white light, although it is not entirely clear from van der Paauw's description whether the absorption by the water was considered in comparing the transmitted energies.

Using a quite similar technic, Bode measured the oxygen consumption of *Fontinalis antipyretica*. Following a 24-hour sojourn in darkness the respiration was measured successively in darkness; in blue light of about 10,000 lux for one-half hour; in red light, presumably of the same intensity, for one-half hour; and in darkness. The radiation was provided by a 2,000-watt lamp with a condensing lens and passed through colored solution filters. Two types of plant material were used, cultured for some weeks prior to the respiration measurements in either red or blue light, using filtered sunlight as the source. Very consistent results were obtained in a series of replicate experiments with each type. In all cases there was an increased rate of oxygen consumption during the periods following the initial dark measurement. The average increases for the blue material were 74% in blue light, 41% in red light, and 46% in the subsequent dark period. For the red material the corresponding stimulations were 64%, 46% and 50%, respectively.

As has been pointed out above, these increases are minimal effects, inasmuch as any photosynthesis which may occur through utilization of the respired carbon dioxide would tend to reduce the apparent respiration. In this connection it may be significant that in all cases the final dark respiration was actually slightly greater than that during the preceding period of red illumination, suggesting that some photosynthesis had occurred. It is interesting also that the red light exerted a somewhat greater stimulation on the plants cultured in red light, whereas the blue light produced the larger effect on the blue material.

Franck and French (91) have studied the influence of high light intensities on oxygen consumption of discs excised from leaves of

Hydrangea and other plants, using the manometric method in the presence of potassium hydroxide. The radiation employed was provided by tungsten filament lamps and filtered through water and glass. By means of a condensing system intensities up to 80,000 lux were obtained. In light of about one-half this intensity, the rate of oxygen uptake was approximately twice that of the preceding dark period; in darkness following the irradiation the rate remained, for a period of 10 to 30 minutes, several per cent higher than that before exposure. The rate in light was not constant, but decreased with time. The velocity of this decay in rate was said to depend upon the previous history of the leaf, on the light intensity and upon the oxygen concentration. Leaves which had been actively photosynthesizing or which had been immersed in sugar solution prior to the measurements were stated to show somewhat greater rates of oxygen consumption which remained constant for a longer time. One rather remarkable result was noted with such sugar-fed leaves: in darkness following a 25-minute light exposure the oxygen consumption was only about 20% greater than that during the initial dark period, whereas subsequent second and third exposures of identical duration and intensity were followed by increases of 87% and 98% above the initial value.

In 1.5% oxygen no light effect was observed. In 60% or 100% oxygen the stimulation of oxygen consumption was considerably greater than in air, whereas the rate of dark respiration was independent of the oxygen concentration over this range. From a comparison of results obtained with continuous light and with intermittent light of much higher intensity, the authors concluded that the light stimulation increases less than linearly with the intensity, at least at high intensities. In view of the inconstancy of the rates of oxygen absorption with time, however, it seems questionable whether such a conclusion is justified by the data presented from this indirect method.

Franck and French stated that experiments with filters of copper sulfate and of potassium dichromate showed large effects induced by red or blue wavelength bands, a result which is regarded as an indication that it is the chlorophyll which sensitizes the reaction. Spectral regions in which chlorophyll absorption is low do not appear to have been tested. The authors have calculated the order of magnitude of the quantum yield for the photoxidation which turns out to be about 0.01 in pure oxygen.

Leaf macerates and leaves killed by immersion in boiling water also showed increased oxygen uptake in light, which resembled closely that observed with living leaves. Because of this finding and that of the quite different relationships between oxygen concentration and oxygen uptake in light and in dark, it was concluded that the effect is a photoxidation unrelated to the normal vital respiration.

Mention should be made also of the work of Godlewski (1879) who compared the amounts of organic matter present in seedlings which had developed, in carbon-dioxide-free air, either in light or in darkness. In several experiments with *Raphanus* and *Zea,* of various ages up to three weeks, no significant and consistent differences were observed between the different light treatments. In similar experiments with wheat, the loss of dry weight was somewhat greater, and the content of water-soluble carbohydrates somewhat less, in plants exposed to a low light intensity than in those grown either in full sunlight or in darkness (175). Shirley suggested that the results of Lubimenko and Karisnev may have been due to temperature differences among the various lots of plants.

e. Suppression of Photosynthesis by Intense Illumination. It has been observed by a number of investigators that under conditions of intense illumination the rate of apparent photosynthesis may be markedly reduced. Where this reduction is great enough to result in a net liberation of carbon dioxide or uptake of oxygen, direct evidence of an alteration of the respiration rate is provided.

In a culture of *Hormidium flaccidum* which had been grown in continuous illumination of low intensity, van der Paauw observed in strong light (presumably furnished by a 150-watt lamp at about 14 centimeters) an oxygen consumption two to four times as great as in darkness. The same result was found repeatedly with this particular culture but could not be obtained at a later date with other cultures. That the cells were capable of assimilation was shown by an experiment at low intensity.

Föckler, who determined oxygen by the Winkler method at hourly intervals, reported a similiar effect for fronds of *Trichomanes radicans* in bright sunlight. The rate of oxygen consumption increased with time of illumination up to about two hours, after which it remained relatively constant for at least two more hours. The maximum rate in light was four to six times that in the preceding dark period. In darkness following exposures of two to four hours the respiration rate fell, at first rapidly and then more slowly; it had not quite returned to the original dark level by the fifth day but had done so by the fourteenth day. Following a one-hour exposure, on the other hand, a further increase in respiration was observed during the first hour of darkness, after which the rate decreased. Although the cells presented an abnormal appearance and a lighter color for a time after the irradiation, they had not been killed; after several days the photosynthetic capacity (in light of moderate intensity) returned. Similar results were found also with portions of thalli of *Laminaria digitata.*

Using the Warburg manometric technic with excellent temperature control, Myers and Burr measured the oxygen uptake by suspensions of *Chlorella vulgaris, C. pyrenoidosa* and *Protococcus* sp. over a wide range of light intensities provided by incandescent lamps. At the highest intensities studied (39,000 foot-candles) the rate of oxygen consumption was as much as three times as great as in the dark. The amount of stimulation, which was less at lower intensities, was relatively independent of the carbon dioxide concentration of the suspending medium, at least within the limits studied, as well as of the cultural history of the cells.

These authors investigated also the time course of the oxygen exchange during exposure to a series of light intensities. On first illumination, oxygen was evolved in all cases; but within several minutes, at intensities of 4,000 foot-candles and greater, the rate of oxygen production began to diminish; at intensities greater than about 12,000 foot-candles, it became negative. On prolonged exposure this absorption of oxygen continued at a fairly constant rate, both the magnitude and duration of absorption depending on the intensity. After one to a few hours the rate of oxygen consumption became smaller, finally approaching zero. At the stage when the decreased rate of oxygen uptake commenced, visible bleaching of the cells occurred; the cells were irreversibly injured at this point, and return to darkness did not result in recovery of the normal respiration rate. This stage appeared to set in after a definite volume of oxygen had been consumed, regardless of the rate of uptake. If the algae were exposed to the radiation for only a short interval and then placed again in darkness, the normal respiration rate was regained after a time. Immediately after turning off the light, however, there was a considerably higher rate of oxygen uptake which was the more persistent the longer the previous exposure had been.

f. Suppression of Photosynthesis by Prolonged Sojourn in Darkness. Kniep reported data from an experiment in which two thalli of *Fucus serratus* were kept in darkness during a period of five months. The plants appeared perfectly healthy at the end of this time, although frequent measurements of the oxygen consumption in darkness showed a continued decrease of respiration, until at the end it amounted to only about one-fifth of the value at the start. The respiration was then measured in diffuse daylight and was even slightly higher than the original level of five months earlier; that is, it showed an approximately 400% increase over the dark rate measured three days before. The thalli were then returned to darkness for two days, after which the respiration of one was measured in darkness, that of the other in light. In darkness there was still apparent about 75% of the stimulation induced by the previous illumination, whereas the second thallus, measured in light, showed a still further increase of 40% over the first light determination.

Föckler kept plants of *Potamogeton lucens* in darkness for 10 days, by the end of which time most of the chlorophyll had disappeared. About 80% of the cells were still viable, as indicated by a plasmolysis test, and the dark respiration was stated to be normal. On illumination by sunlight the oxygen consumption was one and one-half times that in the preceding dark period; on replacement in darkness the respiration returned approximately to the original value.

Indirect evidence, photosynthesis occurring. In addition to the information obtained from experiments in which photosynthesis is experimentally suppressed, circumstantial evidence may be derived from several types of experiments in which the assimilation is allowed to proceed normally. It is under precisely such conditions, of course, that information concerning respiratory changes is of greatest significance for assimilation studies. Indirect evidence of the kinds here considered must of necessity lack conclusiveness, inasmuch as a multiplicity of related or unrelated reactions and physiological processes may influence the over-all changes which are measured.

No attempt will be made in the following discussion to review the vast literature on photosynthesis; a number of citations illustrative of the various lines of evidence should suffice.

a. Evidence from Inconstancy of Photosynthetic Rate at High Light Intensities. In the preceding section there have been considered as direct evidence the cases in which the apparent respiration in light was greater than the respiration in darkness. There are, in addition, a large number of reports of a decreased (although still positive) rate of apparent photosynthesis in light of high intensity.[3] Although such data must be regarded as quite indirect evidence, it seems not improbable that some of the instances cited below differ from those described in the preceding section only in the degree of the respiratory stimulation.

In general two types of information are available: *a*) from experiments in which the photosynthetic rate has been measured as a function of the light intensity at high intensities, and *b*) from experiments in which the time course of photosynthesis has been followed at high intensities. Decreased rates of apparent photosynthesis with increasing light intensities have been observed (73, 103, 125, 140, 141, 195, 226, 281), as well as reduction of photosynthetic rate with time of exposure to light of high intensity (6, 19, 47, 122, 194, 195, 211, 226, 238, 275, 322).

In recent years some workers have referred to these phenomena as "solarization" effects, although the term as introduced by Ursprung in 1917 was used only in connection with starch formation and disappearance.

In general, it may be said that these effects of high intensities are more pronounced in shade leaves or plants than in sun leaves. The sensitivity of many plants can be increased also by shading or darkening for some time prior to the experiment.

Whether the observed diminution of apparent photosynthesis is due to a decrease in the true photosynthetic rate or to an increase in respiration can not be decided with certainty at the present stage of our knowledge. Possibly both factors may be of importance in individual cases. The data of Myers and Burr are consistent with the view that with increasing intensities, there occur a simultaneous progressive inhibition of photosynthesis and a progressive increase of the light-induced oxygen absorption.

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[3] Lest a misleading impression be created, it should be mentioned that in a considerably larger number of experiments the rate of apparent photosynthesis has been found to be quite constant over a wide range of light intensities. While these data in themselves bespeak the absence of any effect of light on respiration, the question always remains whether the constancy would be found also at intensities still higher than those studied.

A number of suggestions as to the possible internal causes of decrease in the true photosynthesis have been advanced: stomatal closure affecting carbon dioxide diffusion, photic or thermal fatigue of the assimilation mechanism, photodestruction of chlorophyll, accumulation of assimilates, and reduction of the functional assimilation surface through phototaxis of the chloroplasts. The diversity of experimental conditions and types of biological material with which the phenomenon has been observed would appear to rule out each of these possibilities as the sole cause of the effect, although all of them may play a rôle in particular instances. It should be mentioned also that in many of the experiments cited the significance of the observed results may be obscured by methodological uncertainties (see 80). For attempts to relate these effects more intimately to the photosynthetic process, reference may be made to Gaffron (98) and to Franck and French.

On the other hand, it can not be denied that these results strongly resemble those, mentioned in the preceding section, in which an increased rate of respiration could be demonstrated directly, the difference appearing to be merely one of degree.

b. Evidence from the Respiration Rate in Darkness after a Period of Illumination. Borodin (29) seems to have been the first to note an increase in the rate of dark respiration, subsequent to a period of illumination, as compared with that preceding it. Rischawi suggested that Borodin's results could be explained, in part at least, through a physical absorption of carbon dioxide in light with subsequent release in darkness. In this connection, although its significance is not clear, mention should be made of the interesting finding that more carbon dioxide could be extracted by a vacuum from leaves (both green and chlorophyll-deficient) after a period of illumination than after a stay in darkness (269).

The criticisms of Rischawi appear to have been satisfactorily refuted by Borodin (30) who confirmed and extended his earlier observations. The general findings were that detached shoots of *Crataegus oxyacantha, Spiraea opulifolia* and *Larix europaea* exhibited a marked increase in rate of respiration, whether measured as carbon dioxide production or as oxygen absorption, following a few hours exposure to sunlight. A greater effect was induced by direct than by diffuse light and much greater by red light than by blue light, although the intensities presumably were not equalized in this comparison. The stimulation was absent, or at most very slight, if carbon dioxide were excluded during the exposure.

Aereboe, who measured carbon dioxide production in darkness by shoots of yellow lupine immediately after excision, found that the rate was markedly influenced by the illumination conditions to which the plants had been subjected during the preceding few days. After a period in bright daylight the respiration was much higher than following a stay in semidarkness. The conditioning of the plants to low or high respiration levels could be reversed by changing from one light environment to the other.

According to Matthaei, the dark respiration rate of detached leaves of *Prunus laurocerasus* after a period of illumination of fairly high intensity was several times greater than that preceding the exposure. Using partly etiolated leaves of *Beta vulgaris,* Meyer and Deleano noted a marked increase in the rate of dark carbon dioxide production after several hours exposure, in the presence of 2% carbon dioxide, to visible electric light of about one-fourth full solar intensity. If carbon dioxide was not provided, only a small, possibly insignificant, effect was found.

Pantanelli (1914) stated that in seven species of marine algae the rate of carbon dioxide evolution in darkness was considerably greater after a period of bright light than after a sojourn in weak light, whereas oxygen absorption usually did not vary greatly under the two circumstances. Hence, a much higher respiratory quotient was found in the algae exposed to the higher intensity. *Valonia utricularis* formed an exceptional case, inasmuch as light treatment occasioned very little change in the carbon dioxide production. The dark respiration of *Enteromorpha compressa* was observed to be about twice as great following a day's exposure to direct sunlight as it was after a day in subdued daylight (120). Spoehr and McGee reported measurements of the carbon dioxide production of detached leaves of sunflower and bean in darkness preceding and following periods of Mazda illumination of a few hours' duration. Of five experiments, one showed a marked increase after illumination, two showed appreciable decreases, while in the other two there were no significant differences. Waller observed a transient acceleration of the carbon dioxide production of detached leaves in darkness after a period of rapid photosynthesis. According to Harder (122), a period of exposure to electric light of 14,000 to 16,000 meter-candles increased the dark respiration of *Fontinalis* by 11% to 61%.

In several experiments with *Hormidium flaccidum,* van der Paauw consistently found that respiration subsequent to irradiation, presumably by a 150-watt incandescent lamp at 13 centimeters distance, was greater than that preceding the light period. Immediately following the illumination the rate of oxygen consumption was increased as much as two-fold; subsequently it declined, rapidly at first, and then more slowly, reaching the original level in about two hours. Parija and Saran observed that the dark respiration of detached *Aralia* leaves was increased by a short exposure to radiation filtered through glass and water, from a 60-watt lamp at 10 inches distance. The stimulation presumably amounted in some cases to 100% or more. An exposure of seven and one-half minutes was stated to result in as large an increase as that for two hours. The data presented indicate that violet light was considerably more effective than white or blue light said to have been of equal intensity; red light was without influence on the respiration. Similar effects were found both with green leaves and with chlorophyll-poor albino leaves, but in either case only with leaves which had been detached from the parent plant for several hours. It was shown also that none of the leaves used was capable of photosynthesis under the conditions of illumination and age at which maximal respiratory stimulations were found.

Mitchell found the dark respiration of entire soybean plants considerably higher following a day of illumination than after an equal period of darkness. Large increases in respiration rate following exposure to sunlight were reported also by Montfort. Stålfelt (281) observed that a period of illumination at 16,000 lux occasioned increases of 18% to 144% in the dark respiration of several species of lichens.

Of 12 species of aquatic plants studied by Gessner (103), six showed an increased oxygen consumption following 40 minutes exposure to electric light of 40,000 lux, whereas six showed a decrease. In view of the large experimental error and the technical difficulties encountered, the significance to be attached to these results appears to be uncertain. Further experiments showed that consistent results could be obtained only when due consideration was had for the duration of the irradiation and of the preceding dark period (104). Following 60 to 65 hours of darkness, exposures of two or four hours to electric light of 40,000 lux resulted in respiin decreases in the subsequent respiration. Four hours of exposure to the arc radiation filtered through glass caused a dark stimulation of approximately 64% in an experiment performed at 25° whereas at 33° a decrease in the subsequent dark respiration was noted. After six hours of violet light, obtained by means of a methyl violet solution filter, the rate of dark carbon dioxide production was unaffected in one experiment but appreciably decreased in a second.

In contrast to the numerous observations of increased respiration following irradiation are those of a limited number of experiments with *Aspidistra,* which revealed a much smaller rate of dark carbon dioxide production after an illumination period (100). In view of the inconstancy of the respiration rates in these, as well as other experiments performed at the same time, the validity of the results appears questionable.

In recent years several workers, using improved technics, have observed irregularities in the rate of gas exchange immediately after a transition from darkness to light, or *vice versa* (8, 9, 21, 81–83, 98, 180, 181). Numerous suggestions of more or less similar "anomalies" are to be found also in the older literature on photosynthesis. The significance of these results is not entirely clear, as yet; in any case, the effects are of much smaller magnitude than are those described above.

On the other hand, many investigators who have measured, in connection with studies of photosynthesis, the respiration in darkness before and after illumination have never noted after-effects of the irradiation. In view of the possibility that many as yet unappreciated factors may influence the respiration response to light, it seems reasonable to expect that either positive or negative results could be obtained if such factors were not controlled. For this reason the reviewer feels that, of equally careful experiments, those which demonstrate positive effects are of much greater evidential value.

Some additional lines of information should perhaps be included in this section, although, admittedly, they may prove to have little bearing on the question concerned.

Saikewicz, who measured the carbon dioxide production by intact roots of maize plants, found a greater respiration rate during the day than at night; a decrease in rate was noted also when the plants were moved from bright sunlight into diffuse light. Similar experiments have indicated that a light-induced periodicity in the root respiration occurred only after the plant had been kept for some days in conditions of low light intensity (3).

A number of workers have compared the respiratory activities of plants cultured under different intensities of light. In *Teucrium scorodonia,* Rosé found the highest rates of carbon dioxide production, on a fresh weight basis, in plants grown at intermediate intensities; this was true also for older plants of *Pisum sativum,* whereas among younger plants those cultured at the highest intensity showed the greatest respiratory rate. The respiration rate, on a fresh weight basis, was considerably less in partially etiolated leaves of *Raphanus sativus* which had developed in light of low intensity than in those grown in higher intensities (43).

Hicks and Panisset, who cultured *Lemna* at various intensities of 350 to 1,400 foot-candles, reported that the rate of carbon dioxide production per frond was greater the lower the light intensity. In similar experiments with *Lemna minor,* the respiration rate, calculated on the basis of leaf area, increased with increasing light intensity of the previous culture period, but on a dry weight basis the reverse was true (319). According to Chia, anthocyanin-containing plants of *Amaranthus* which had been grown under intensities of artificial light of 31, 220 and 431 foot-candles showed relative respiration rates of 100, 138 and 163, respectively, as compared on a fresh weight basis. Sargent found the rate of oxygen uptake per unit volume of *Chlorella pyrenoidosa* cells cultured at high light intensity to be nearly twice that of cells grown at low intensity. The difference in rate of carbon dioxide production was considerably less, so that the average respiratory quotient for the "sun" plants was 1.1, whereas that for the "shade" plants was 1.6. Iuracec compared the carbon dioxide production by leaves of bean plants which for some days previously had been cultured in direct sunlight or in shade. The respiration of the sun leaves was 80% to 90% greater when calculated on the basis of area, fresh weight or water content, 40% greater on a dry weight basis, and 106% greater per leaf.

c. Evidence from the Compensation Point. The term "compensation point" was introduced into plant physiology by Plaetzer in 1917 to designate the light intensity at which assimilation and respiration rates compensate each other with a resultant zero gaseous exchange. The compensation point as thus defined has proved to be an extremely valuable concept, especially in ecological problems. It may be mentioned parenthetically, however, that it lacks precision to the extent that the material under consideration is not definitely circumscribed. Thus, a chloroplast, a cell, a leaf and a shoot, as well as the entire plant itself, will in all probability have quite different compensation points. Further complication arises through the possibility, occasioned by differences between respiratory quotient and photosynthetic quotient, that under some conditions there may exist separate compensation points for oxygen and for carbon dioxide. Obviously, the compensation point will be influenced by all those factors which affect either photosynthesis or respiration. Variations of the compensation point thus afford evidence of changes in one or both of these processes.

There is abundant evidence that the compensation point is influenced greatly by the previous illumination intensity to which a plant has been exposed. In some cases it has been possible experimentally to effect more or less marked alterations of the compensation point by culturing in light of different intensities (62, 76, 121). The compensation point is higher after a regime of more intense illumination. Numerous studies have demonstrated, furthermore, that the compensation point of sun plants or sun leaves is, in general, higher than that of shade plants or shade leaves (*e.g.*, 33, 34, 76, 121, 129, 177, 260, 280a).

From such evidence alone it is, of course, not possible to definitely ascribe the compensation point differences either to an altered respiration rate or to an altered assimilation rate. A number of investigators have shown, however, that the respiration rates of shade plants or shade leaves are, by and large, appreciably smaller than those of corresponding sun plants or sun leaves (*e.g.*, 4, 34, 101, 126, 141, 177, 189, 260, 280a). (Compare also the several examples, given in the preceding section, of lower respiration rates in plants cultured under low light intensities.)

It seems altogether likely, therefore, that the influence of illumination conditions on the compensation point is due, at least in part, to an effect of light on the respiration rate.

A quite different type of indirect evidence was utilized by Noddack and Kopp, who worked with *Chlorella pyrenoidosa.* From a consideration of the relationships between the rate of apparent photosynthesis and the light intensity at different temperatures, it was calculated that the respiration was diminished in light, under the conditions employed (15-minute exposure to low intensity light of λ6500 Å).

EFFECTS OF NON-VISIBLE RADIATION

Infra-red Radiation

With relatively few exceptions investigators have recognized the possibility of an indirect temperature effect due to absorption of infra-red radiation and in most instances have made some attempt to eliminate it. There appears to be no evidence of direct specific infra-red effects.

Ultra-violet Radiation

Cook and Stephenson measured the oxygen consumption by *Escherichia coli* immediately after exposure to radiation from a quartz mercury arc. In the presence of glucose, acetate or lactate, the total amount of oxygen absorbed for a given amount of substrate was uninfluenced by prior irradiation in dosages great enough to reduce the number of viable organisms to less than 0.3% of the control. The initial rate of oxygen uptake, however, was decreased by approximately 58% in the irradiated cultures. With formate as substrate, the initial rate following exposure was only about one-tenth, and the total amount of oxygen absorbed was only about one-half of that of the control.

Giese studied the influence of ultra-violet radiation (consisting chiefly of λ 2537 Å) on the oxygen uptake of the luminous bacterium *Achromobacter fischeri,* using the Warburg technic (105). After small dosages of radiation (of the order of 1,000 ergs/mm.^{2}), the respiration rate was unaffected for several hours but eventually showed a decline. Larger dosages (up to 64,000 ergs/mm.^{2}) were followed by earlier and more pronounced decreases in the rate of oxygen absorption, the effects being proportional to the dosage. Since the respiration (in presence of glucose and/or peptone) was affected similarly whether the cells were irradiated in the presence or absence of the substrate and as no effect was produced by irradiation of the medium alone, it was concluded that the radiation acted directly upon the organism. The relationship found between concentration of glucose supplied and rate of oxygen uptake suggested that the glucose-activating dehydrogenases were selectively inactivated. Giese reported that if the cultures were shaken in absence of nutrients for long periods prior to irradiation, a respiratory stimulation could be observed following exposure (106). Thus, after an irradiation of two and one-half minutes at about 70 ergs/mm.^{2}/sec., the rate of oxygen consumption was approximately 40% greater than that of the control; a 10-minute exposure resulted in an increase of about 100% but this was not maintained for as long a time. Cells irradiated shortly after washing from nutrients did not manifest the ultra-violet-induced stimulation but showed a decline in respiratory rate, as previously found.

The influence of ultra-violet radiation on respiration and fermentation of yeasts has been studied by a number of investigators. A considerable portion of these experiments, performed with impure cultures, in complex media, by inadequate technics or without regard for effects on growth and viability, are very difficult to evaluate.[4]

Very striking claims of increase in fermentation rate following irradiation have been made (65–67, 87, 169). It was increased 22% by exposure to radiation of λ 2,000–2,500 Å for five minutes at 15 centimeters from a quartz mercury arc of 1,000 Hefner candles intensity (35), and small transient increases in the rate of anaerobic carbon dioxide production were noted after short periods of irradiation by a quartz mercury arc (329). Longer exposures resulted in a slight inhibition. Hinrichs also found short exposures to result in increased fermentation, whereas more prolonged irradiation was inhibitory. Others have concluded from their experiments that ultra-violet irradiation resulted in an increased fermentation rate and higher fermentation efficiency (221, 222), but these conclusions have been severely criticised (292, 326).

Gronchi measured carbon dioxide production during and after exposure of glucose suspensions of *Saccharomyces cerevisiae* to a quartz mercury arc, using filters to isolate radiation of 2,200–3,800 Å and 3,650 Å (111). During the exposure the fermentation rate was greater than in the untreated control, but following the treatment the rates dropped to values much lower than that of the control. The inhibition produced by the 2,200–3,800 Å radiation was evident even 48 hours later.

Others have reported that short exposures to ultra-violet radiation stopped fermentation (186, 187), or decreased it (1, 278,

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[4] Much of the earlier literature has been critically discussed (292, 326).

292, 293). No evidence of stimulation was ever observed by these authors. A temporary decrease, possibly followed by a slight increase, in rate of alcohol production, was observed on one occasion (10).

The influence of a number of spectral regions, obtained with a quartz mercury arc in conjunction with various filters, on the anaerobic carbon dioxide production by baker's yeast, has been studied (248). The measurements, made during the second hour of irradiation, showed wave-lengths longer than 3,000 Å to be without effect on the fermentation rate, whereas marked inhibition was produced by radiation between 3,000 and 2,500 Å. The inhibition was unaccompanied by general injury to the cells, so far as could be determined from the stainability by methylene blue.

Oster measured the oxygen consumption of yeast immediately after exposure to various ultra-violet lines of the mercury spectrum and observed no effect on respiration unless the irradiation had been sufficient to damage the cells, in which case the oxygen uptake was decreased. Tang found that exposure of *Saccharomyces wanching* to the radiation (of unspecified intensity) from a quartz mercury arc decreased the subsequent dark respiration, the degree of inhibition increasing with the duration of the irradiation period. The aerobic respiration of *Saccharomyces cerevisiae* was stated to be relatively unaffected by ultra-violet radiation of wavelength 2,650 Å (5). A 50% increase in oxygen uptake due to ultra-violet raying has also been observed (286).

The problem of stimulation of yeast respiration by ultra-violet irradiation was studied also by others, using the manometric method (85). In several experiments exposure to radiation of unspecified intensity from a quartz mercury arc increased the subsequent respiration rate by amounts up to 157% above the untreated control. Under the conditions employed the maximum stimulation occurred after five minutes of irradiation; exposures of 10 minutes or longer depressed the rate of oxygen consumption below that of the control. Increases in respiration were not accompanied by more rapid cell reproduction; rather, the raying resulted in a retarded multiplication rate. Evidence was obtained also that the respiratory stimulation persisted for at least 24 hours after treatment, although these experiments are less clear-cut. Additional study showed, however, that the results could not be obtained consistently; in many cases depression of respiration was observed under the conditions which had originally produced stimulations.

The effect of ultra-violet irradiation on oxygen uptake of *Saccharomyces cerevisiae* has been investigated also by Giese (106). A Sterilamp, emitting principally $\lambda 2{,}537$ Å, was used as the source; the incident intensity was approximately 70 ergs/mm.^{2}/sec. The relatively low rate of respiration of washed cells suspended in buffer solution was markedly increased after short exposures. Thus, in one experiment treatments of 1.25, 5 and 10 minutes, respectively, resulted in increases of approximately 20, 80 and 200%. The increased rate of oxygen uptake, which, incidentally, was never greater than that of non-irradiated sugar-fed cells, was maintained practically constant for at least two hours and in other experiments was evident at least 12 hours later. An exposure of 20 minutes gave an even larger initial stimulation but was not maintained. The reproductive capacity of the cells was very greatly diminished by even the 10-minute irradiation.

Cells furnished with glucose in concentration high enough ($>0.1\%$) to produce the maximal rate of oxygen uptake in the controls, showed no stimulation after ultra-violet exposure but, instead, exhibited a respiratory rate smaller than that of the non-irradiated culture. Stimulation by ultra-violet was elicited, however, in the presence of suboptimal amounts of glucose (0.05%).

Apparently inconsistent results were obtained on the addition of glucose to cultures which had been irradiated some hours earlier in its absence; in one case the sugar produced a marked increase in rate of oxygen consumption, whereas in another experiment no effect was observed. The cause of this discrepancy is not evident; it would appear that the factors governing the respiratory response to radiation are not entirely appreciated, and this probably accounts for the discordant findings of various investigators. It is interesting to compare the results of Giese with those of Rubenstein on the effect of visible radiation on respiration of *Sarcina lutea,* which have been mentioned above. In the work of the former the possible influence of the temperature was not investigated. Giese concluded that the respiratory stimulation induced by ultra-violet irradiation is not due, in any considerable measure, to the liberation of respirable substrate from the cells, but rather to the production of catalytically active substances which enhance the capacity of the starving cell to oxidize its contents. In experiments with the mycelium of *Neurospora crassa,* no evidence of ultra-violet stimulation was observed.

Pasinetti and Grancini reported that several minutes exposure to ultra-violet radiation had no marked effect upon the subsequent gaseous exchange of *Corticium rolfsii, Sclerotium delphinii* and *Alternaria brassicae.*

Bonnier and Mangin (27) found different respiratory quotients for several green plants when measured in darkness and when measured during exposure to ultra-violet radiation obtained from sunlight by means of filters. It is possible, as the authors believed, that some photosynthesis was taking place so that the results are not at all conclusive but merely suggestive of a possible effect of ultra-violet radiation on respiration. Masure reported two experiments in which the oxygen uptake of etiolated pea seedlings was measured before, during and after exposure to a band of radiation, of unmeasured intensity, from about 3,334 to 3,650 Å. Certain large irregularities in the results are attributed by the author to heating effects, and it appears possible that the observed respiratory stimulations may in reality have been due to similar causes.

Wynd, Fuller and Reynolds subjected plants to successive short daily doses of mercury arc radiation minus the infra-red longer than 1.4 μ, in addition to the normal solar illumination. In one experiment the carbon dioxide production of tomato plants on the day following five weeks of daily 10-minute exposures to radiation (lacking the ultra-violet shorter than 2,894 Å) was 31% greater than that of the controls; the treated plants also showed a higher catalase activity, whereas oxygenase and peroxidase activities were practically the same as in the controls. On the next day no marked respiratory stimulation could be demonstrated, but the catalase activity was still quite high. Plants which had received radiation including the shorter ultra-violet, exhibited no appreciable increase in respiration, although the catalase activity was even greater than in the plants mentioned above. A somewhat similar experiment with beans gave quite different results. In the irradiated plants, all of which showed injury, the peroxidase activity was very markedly increased over that of the controls, whereas the catalase activity and respiration rates were not consistently different in the two sets.

In a second experiment with tomatoes, radiation, including the shorter ultra-violet of much higher intensity, was supplied in 15- or

30-minute daily doses over a four-day period. Evidence of respiratory stimulation persisting for at least four days after cessation of irradiation was obtained, but interpretation of the results is complicated by the marked injury suffered by the plants. In general, the peroxidase activity of these plants was higher, the oxygenase activity lower than in the controls; the catalase activity which on the day after termination of the irradiation was lower than in the controls, increased day by day until on the fourth day the value was much higher than in the untreated plants.

Chia observed that exposure of *Amaranthus cordatus* to an unscreened Cooper-Hewitt mercury arc resulted in increased oxygen consumption as compared with unirradiated control plants. Fifteen- and 30-minute exposures gave increases of 10% and 12% respectively, whereas an hour's treatment produced only a 4% increase. Following daily exposures of three to five minutes, continued over a period of three weeks, respiratory stimulations of 20% to 23% were found.

In the experiments of Gessner (104) an apparent decrease in oxygen consumption was observed during exposure to long wavelength ultra-violet radiation, but in this case, too, it is quite likely that the ultra-violet or the small amount of red light which was also present may have permitted some photosynthesis.

The results of Föckler on excised roots and of Ranjan with green leaves have been described in preceding sections.

Further use of ultra-violet radiation as a tool would appear to be rather promising in view of the finding that by such irradiation photosynthesis of *Chlorella* could be entirely inhibited for at least seven hours without any appreciable effect on the dark respiration (7).

In summarizing briefly the results obtained with ultra-violet it may be said that, so far as the respiration of bacteria and yeasts is concerned, such radiation has been found to be either inhibitory or without influence; no claims of stimulatory effects have yet been made. The available reports dealing with yeast fermentation are approximately equally divided between claims of stimulations and of inhibitions. The best work on higher plants indicates that more or less clear-cut stimulations may be induced by irradiation. The findings from the experiments in which long periods of treatment have been employed are difficult to assess, however, since many secondary effects must be taken into consideration.

X-Radiation

The influence of x-rays on respiration and fermentation of aqueous suspensions of baker's yeast has been studied, and manometric measurements made 30 to 60 minutes after exposure showed no significant differences between treated and control cultures (317). Oxygen consumption of *Staphylococcus aureus* also was unaffected by x-irradiation, even though the reproductive capacity of the cells was greatly diminished by the treatment. Similar results were obtained with suspensions of beer yeast (318).

According to von Euler, the rate of oxygen consumption by yeast was increased about 40% after x-irradiation, but returned to normal on the addition of sodium arsenate. The increase in rate was partially nullified by addition of yeast extract, which however, had no influence on respiration of normal cells.

Schneider found the fermentation rate of yeast reduced by x-irradiation (262), but in the presence of dyes no influence could be demonstrated (263). The results of Lossen and Schneider were rather inconclusive. According to Zeller, the rate of anaerobic carbon dioxide production by yeast following x-irradiation decreased at first, then increased and finally returned to the level of the unexposed control. His results suggested that misleading conclusions might be reached unless care were taken to observe the entire course of events subsequent to irradiation.

Gronchi observed increases of the order of 10% to 40% in rate of yeast fermentation, both during and after exposure to x-rays (110, 112). The magnitude of the stimulation was said to be proportional to the intensity of the radiation within the range employed (dosages of 300 to 2,000 roentgen units during a period of 45 minutes). Greater effects were produced by shorter (average = 0.16 Å) than by longer (average = 0.37 and 0.55 Å) wavelengths. Others have reported 5%, 20% and 35% increases in rate of oxygen uptake following x-irradiation of yeast suspension for 15, 30 and 60 minutes, respectively (85).

An increased rate of gaseous exchange following x-irradiation of *Corticium rolfsii, Sclerotium delphinii* and *Alternaria brassicae* has also been observed (228).

Petry measured the gaseous exchange of pea and wheat seedlings during the 24-hour period immediately after a 15- to 30-minute exposure to x-rays. Both carbon dioxide production and oxygen consumption were reduced by about 10% in comparison with the untreated controls. Five to eight days later the respiration was only 40% less than that of the controls, even though marked growth derangements were manifest. The carbon dioxide production of *Helianthus annuus* seedlings during a nine-day period subsequent to x-irradiation of the seeds was investigated and both the respiratory rate and the growth rate, as compared with controls, were found to be decreased (142).

Francis measured the rate of carbon dioxide production at 6, 28 and 52 hours after exposure of 24-hour-old wheat seedlings to various dosages of x-rays up to 13,560 roentgens. The rate of respiration per plant was depressed in all cases except for the earliest measurement of the plants receiving the smallest dosage (565 roentgens), which showed a small stimulation. The extent of the inhibition was dependent upon the dosage. Growth was also depressed by the treatment so that, in comparison with the controls, the exposed plants exhibited a slightly greater respiratory rate on the basis of linear growth of the seedlings, a somewhat lower rate on the basis of dry weight and, at the earliest measurement, a greater respiration on the basis of fresh weight.

Chesley exposed 18-hour-old wheat seedlings to doses of γ- and x-rays sufficient to decrease the subsequent growth by as much as 40%. Growth, as measured by fresh weight, was markedly reduced even in the first few hours after treatment, whereas the oxygen consumption (measured manometrically) per plant was not significantly different from that of the controls. Respiration expressed on unit weight basis was, therefore, greater in the irradiated seedlings. During the period from 24 to 48 hours after exposure the oxygen uptake closely paralleled the growth: the respiration per plant was lower in the treated seedlings, but on a weight basis was nearly identical in the two sets. In an experiment in which the seedlings were kept at 6° during the 48 hours immediately following irradiation, no growth occurred, and at the end of this period the respiration (measured at 26°) was equal in treated and control plants whether calculated per seedling or per gram. These results indicate that the effects on respiratory rate are expressions of the decreased growth rather than its cause, and Chesley concluded that the primary influence of the radiation is not upon the respiratory mechanism itself.

Shull and Mitchell obtained indications of increased sugar content and respiration rate of corn seedlings after irradiation with small dosages of short wavelength x-rays.

Carbon dioxide production of sprouted bulbs of *Narcissus tazetta* has been measured at various intervals following exposure to an x-ray dosage of 3,500 roentgens. The results of separate experiments were rather inconsistent, and no general conclusion appears to be warranted. Thus, of seven sets of plants measured one-half hour after exposure, the carbon dioxide output on a fresh weight basis was less than that of the controls in four, greater in two, and equal in one. Of three other sets measured five hours after irradiation, one showed an increase, one a decrease, and one no change in the rate of carbon dioxide production as compared with the controls. Results at later periods were complicated by differences in the weight and height changes.

Bersa determined the rate of oxygen consumption by one-centimeter long root tips excised at intervals subsequent to exposure of soaked *Vicia faba* seedlings to x-rays. Roots excised within less than an hour after treatment showed a slight respiratory stimulation; at six hours no difference was found between irradiated and control roots. After intervals of one, two or three days the irradiated roots were markedly shorter and thicker than those of the normal seedlings, and the rate of oxygen uptake, on a fresh weight basis, fell ultimately to about one-half that of the controls.

To recapitulate the effects of x-radiation, no influence on bacterial or yeast respiration has been reported. With the exception of the somewhat uncertain results of Schneider, yeast fermentation has been found to be stimulated to some degree. The findings with higher plants are in fairly good agreement, generally showing decreases in respiration, although small and transient stimulations may be produced by low dosages. The inhibitions, at least, appear to be related to more direct influences of the radiation upon other physiological processes rather than primarily due to action upon the respiratory mechanism.

γ–*Radiation*

As the studies dealing with the effects of γ–radiation on respiration, fermentation and metabolism of plants have been admirably reviewed in a monumental treatise (285), they need not be considered here.

Hertzian Radiation

Benedetti measured the anaerobic carbon dioxide production by yeast following exposure to a high-frequency electro-magnetic field. Either increases or decreases in fermentation rate were found, depending upon the culture medium, the duration of treatment and the frequency used. Others have claimed that the fermentative rates and capacities of a number of yeasts, molds and bacteria were markedly increased by electromagnetic radiation of wavelengths between 1.8 millimeters and 120 meters (168). Stimulation of carbon dioxide production by yeast after exposure to radiation of wavelengths 1.7 and 1.88 meters has also been reported (237). Claim of stimulation of bacterial fermentation is made in a patent issued to Ternion A.-G. (294).

MECHANISM OF THE EFFECT OF RADIATION ON RESPIRATION

As to the mechanism by which the respiration rate is influenced by radiation relatively little definitive information is available. Respiration is the expression of a congeries of delicately balanced processes and hence is exceedingly sensitive to internal and environmental conditions. It seems altogether probable that no single explanation will be found for the effects observed in widely diverse types of living material and produced by various kinds of radiation. In many instances the effect upon respiration is doubtless a secondary result of some other process or processes which are more directly influenced by the irradiation. This would appear to be especially true of non-visible radiation.

The influence of radiation upon respiration and fermentation of yeast has been shown, in some instances at least, to be of an indirect nature. Stimulation could be induced in non-treated suspensions by addition of the cell-free culture fluid from ultra-violet irradiated suspensions and, to a lesser degree, even by addition of culture medium or of glucose solution which had been rayed in the absence of the yeast. The authors concluded that irradiation of yeast increases the production of a substance (formed also by non-irradiated yeast) which stimulates the respiration (86).

Norris and Ruddy believed that radiation acts by killing a portion of the cells which thereby liberate the stimulatory substance. In the luminous bacterium, *Achromobacter fischeri,* Giese (105) found, however, that extracts from irradiated bacteria or from eggs or sperm of *Arbacia* had no specific stimulatory effect on the oxygen uptake, but acted merely as nutrients. Numerous studies by other workers also have demonstrated that irradiation of the culture medium alone can have a marked effect upon the growth and metabolism of cells subsequently inoculated into it. The primary action of the radiation seems to be chiefly on the organic constituents, usually carbohydrates, of the medium.

A question which has oftentimes been raised is whether the changes in rate of gas exchange observed to result from irradiation with light have any relation to the normal respiratory processes. In this connection the view has been advanced that the increased absorption of oxygen or emission of carbon dioxide is due to a photochemical reaction entirely unconnected with respiration. In green plants such a photochemical reaction might be bound up with the photosynthetic mechanism (*e.g.*, 95, 98). The depressant effect of high oxygen concentration upon the rate of photosynthesis in intense light has been interpreted by some as evidence of a photoxidation.

It is, of course, well known (*e.g.*, 71, 78, 235) that radiation of various wavelengths promotes chemical transformations, *in vitro*, of many substances which occur in plants. Some of these reactions are accompanied by absorption of oxygen or liberation of carbon dioxide or both. There are many examples also of *in vivo* chemical reactions which are directly or indirectly influenced by radiation. Since, for the most part, such reactions have not been studied in relation to the gaseous exchange of the plant, a discussion of this literature will not be attempted here. On the other hand, the destructive effects (most frequently observed as a bleaching of the chlorophyll) occasioned by exposure of green plants to light of high intensity which have been noted by many workers, have in a number of cases been accompanied by increased oxygen uptake. It appears altogether reasonable then, that photochemical reactions may be directly involved in some of the experiments cited above. Whether any of these instances can be explained entirely on such a basis is uncertain. If this were the case, it might be expected that the amount of excess "respiration" would be related to the amount of photolabile cellular material present. Such a relationship is indeed indicated by the data of Myers and Burr who suggest that photoxidation may depend upon absorption of light by the chlorophyll. Possibly this hypothesis could be tested by a comparison of cells with different chlorophyll contents or by a comparison of wavelengths absorbed to different degrees by chlorophyll; the influence of added sugar or of other respirable materials on the light effect might be worthy of investigation also. Other results (91) mentioned above, indicated that greater photoxidation effects were obtainable with sugar-enriched leaves, but the experimental material employed appears to have been unfavorable for quantitative studies.

A photochemical reaction which conceivably may be of widespread occurrence is nitrate reduction (173a, 316). As oxygen is produced in this case the result would be a diminution of the apparent respiration (as measured by oxygen consumption) in light. There is fairly general agreement that the reduction of nitrates by green plants is enhanced by irradiation, a circumstance which may be of significance in this connection, although it is not established that the effect is direct.

Of considerable interest with regard to the mechanism involved is the oft-repeated observation of a respiratory effect in darkness following irradiation. Obviously, the overall reaction can not strictly be termed photochemical, inasmuch as it persists long after absorption of radiant energy has ceased. It is to be regarded, rather, as a complex process in which some primary photochemical step, often requiring a relatively small amount of energy, sets in motion a long-continuing train of events.

Turning now to the possible effects of radiation upon the respiratory mechanism itself, it is well known that the activities of many enzymes[5] are influenced profoundly by diverse forms of radiation. The activities of *in vitro* preparations are usually diminished particularly by γ-rays, x-rays and ultra-violet (for literature see 78, 218, 235, 264, 326). Some coenzymes, too, have been found to be destroyed by ultra-violet irradiation (*e.g.*, 309, 315).

Several examples of enzyme inactivation by visible radiation have been reported: *e.g.*, catalase (171, 172, 220, 331), peroxidase (137), succinodehydrogenase (236), yellow enzyme (295), sucrase (72, 137, 139), diastase (109, 213).

On the other hand, numerous investigators have noted increased activity of enzyme preparations following exposure to visible radiation: *e.g.*, catalase (58, 149, 206, 207), peroxidase (14, 220), xanthine oxidase (16), various dehydrogenases (161, 163, 171, 306), amylase (56, 58, 109, 134, 203, 207, 210, 213), sucrase (107, 108, 201, 207, 209, 210), pepsin (56, 149), trypsin (149), papain (18), proteases (58, 202, 209, 210), lipase (58, 149, 204, 209, 223), urease (205). In a few instances ultra-violet, infra-red or x-ray irradiation also has been found to increase enzyme activity *in vitro*.

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[5] No distinction is made here between preparations containing only the enzyme and systems in which the substrate also is present. A number of workers have shown, however, that the effects of irradiation may differ in the two cases.

A special case of light activation is that produced by polarized light (9a 9b, 181a, 267a). Others have been unable to confirm this finding (47a, 143a, 213).

As has been pointed out by numerous investigators, conclusions from *in vitro* studies should be applied only with the greatest reserve to *in vivo* reactions. Experiments relating to alterations of enzyme activity of plants following irradiation *in vivo* are less numerous. Green found an increase of diastatic activity when living leaves were exposed to infra-red, red or blue radiation and concluded that the treatment promoted the conversion of a zymogen into the active enzyme. Others found a 30% decrease in the catalase activity of yeast which had been exposed to sunlight for 30 minutes (308), and later that irradiation by a quartz mercury arc resulted in two- to eight-fold increases in the catalase activity (307). Abramov also noted augmented catalase activity of yeast after exposure to ultra-violet radiation, whereas sucrase, zymase, maltase and proteolytic enzymes were inactivated in various degrees. Yamafuji observed increases in catalase activity up to 2,400% after exposure of various yeasts to radiation from a quartz mercury arc. The effect was due chiefly to the shorter ultra-violet region. The increased acidity following illumination of the light-sensitive seeds of *Nicotiana tabacum* and *Verbascum thapsus* was interpreted as evidence of enhanced lipolytic activity (99). Burge and Burge, who transferred *Spirogyra porticalis* from a frozen lake to higher temperatures, observed a more rapid increase of catalase activity if the plants were illuminated.

Illuminated detached leaves of *Allium tuberosum* and of *Mangifera indica* were observed to have a greater catalase activity than those which had been kept in darkness (246). Similar results were obtained with *Croton* leaves (242). If sugar were supplied to the leaves, however, no difference in enzyme activity was found. These results were believed to indicate a relationship between hexose production and the catalase activity of the leaf. Catalase and peroxidase activities of rice seeds were diminished by exposure to sunlight (155), whereas no significant difference in catalase activities of *Raphanus sativa* seedlings germinated in dark or in light was noted (60). Ascorbic acid oxidase activity was less in seedlings germinated in sunlight than in those developed in darkness (212), and when soaked seeds of tau-sagyz were exposed to radiation from a quartz mercury arc and the catalase activity of the seedlings measured after two days of germination, the treatment resulted in increases up to 16-fold.

Fuller subjected bean and tomato plants to successive short daily exposures to ultra-violet radiation sufficient to cause serious injury. At the end of seven days of treatment the amylase, invertase, peptase and catalase activities, on a fresh weight basis, were markedly higher in the irradiated plants than in the controls. Slight increases in the activity of amlyase and peptase were observed also in mycelia of *Fusarium lini* which had received ultra-violet irradiation just sufficient to kill. Other investigators have noted increased enzymatic activity attendant upon the killing of plant tissues by ultra-violet irradiation, but these responses do not appear to be specific, since many other methods of causing death have the same result.

Harker reported decreased invertase activity of yeast following exposure to γ-radiation (plus some β-radiation) from radium.

The remarkable reports of increased diastase activity in corn and potato plants days or even weeks after exposure of the seeds and tubers to visible or infra-red radiation (274), are difficult to evaluate, since neither the development of the plants nor the transmissions of the filters employed were described.

In only a few of these studies has respiration been measured along with enzyme activity. Although the rôle played in plant respiration by the known oxidation-reduction enzymes and by the carbohydrases is at present very imperfectly understood, it does not seem unreasonable to expect that changes in the activities of such enzymes might be reflected in an altered gaseous exchange. Giese concluded that the decline in rate of oxygen consumption, observed by him to follow ultra-violet irradiation of *Achromobacter fischeri*, could be interpreted best as due to a decrease in the concentration of dehydrogenases (105).

In the experiments in which attempts have been made to correlate the effects of radiation upon respiration and on enzyme activity of the plant, it has usually not been possible to demonstrate a close relationship. Von Euler and Laurin, who noted a 30% decrease in catalase activity as the result of solar irradiation of yeast, found at the same time only a 5% reduction in fermentation capacity.

Ranjan and Mallik observed that catalase activity, found to be influenced by illumination, was not consistently correlated with the respiration rate. In wheat plants, also, the respiration rate was not correlated with the catalase activity, which was increased by low temperature storage in darkness but decreased by similar storage in light (70). Schröppel reported that the increase in respiration rate resulting from illumination of light-sensitive tobacco seeds preceded by some hours the increases in catalase and peroxidase activities. Pal apparently observed an increase in the value of the respiratory quotient on illumination of germinating *Helianthus* seeds and concluded that light decreased the rate of conversion of fat to carbohydrate (224). The same conclusion was reached by Brown.

Some experiments concerning oxidase activities of plants following ultra-violet irradiation (325) have been mentioned in a preceding section. Regrettably, interpretation of these experiments is rendered difficult by the failure of the authors to specify the basis on which the enzyme activities were compared or to present information which would permit an assessment of the rôle played by the radiation injuries encountered. From the data presented, however, the activity of none of the enzymes studied appears to be quantitatively correlated with the respiration rate.

Petry was unable to detect any effect on catalase or peroxidase activity of legume seedlings which had been x-rayed under conditions presumably identical with those which resulted in a 10% decrease in respiration. In seedlings of *Helianthus annuus* from x-irradiated seeds, the rate of carbon dioxide evolution and the catalase activity were decreased, while the oxidase activity was unaffected (142).

Among the less direct mechanisms through which the respiration rate could conceivably be affected by radiation may be mentioned those which influence (*a*) the amount of available respiratory substrate and (*b*) the rate of movement of materials in the plant or the exchange between plant and environment.

In 1876 Borodin suggested, as have also a number of later investigators, that the observed stimulations were due to increased concentration of respiratory substrate made available by photosynthesis during the illumination period. This appears to be a likely explanation of some of the findings noted, although direct proof is lacking. On the other hand, such an explanation would not seem to apply, without some modification, to the results of Palladin, who measured the carbon dioxide production of leaves detached from etiolated *Vicia faba* seedlings and floated on sucrose solutions. The respiration rate in darkness after a few days of exposure to daylight was more than twice as great as that of leaves which had been maintained continuously in the dark. Increased respiration was noted also after sojourn in blue or in yellow light. No consistent difference between the results obtained with the two spectral regions could be established; no effort to equalize the intensities seems to have been made, however. Possibly an intensity difference accounts for the greater stimulation produced by white light than by the colored light.

The above explanation based on substrate accumulation due to photosynthesis is also not supported by experiments with *Hormidium* and *Oocystis* (303). Respiratory stimulations of approximately the same degree were found consistently after periods of illumination, whether the carbon dioxide concentration was high or was kept at a very low level by the presence of potassium hydroxide. Parija and Saran found respiratory increases following brief illumination of detached leaves shown to be incapable of appreciable assimilation. In other experiments, too, there was noted a respiratory stimulation after exposure, even though the rate of photosynthesis was not great enough to overbalance the carbon dioxide excretion (241, I). Furthermore, as pointed out, for example, by Stålfelt (281), a decrease in concentration of photosynthate rapid enough to account for the frequently observed swift decline in respiration rate subsequent to illumination would also require explanation.

In addition to the possible rôle of photosynthesis in increasing the amount of respiratory substrate, enzymes such as diastase and invertase, of which the activity has been shown to be influenced by radiation, are doubtless important in determining the concentration of respirable materials in the cell. A diurnal periodicity in invertase activity of tobacco plants, manifested by a high synthetic activity during the day and a high hydrolytic activity at night, has been reported (289). In one of the two varieties studied the respiration rate also showed a maximum during the day and a minimum at night. Daily periodicity of respiration and of enzyme activity in plants has been noted frequently; however, light may not be the factor which governs this phenomenon (see *e.g.,* 124, 256).

Although there has accumulated considerable evidence that secondary carbohydrate transformations in plants may be influenced by radiation, such effects have usually not been shown to be related to the respiratory process. A notable exception is the demonstration that brief illumination of starved detached *Aralia* leaves resulted both in marked stimulation of the subsequent dark respiration and in two- to seven-fold increases of the reducing sugar content (227). Preliminary experiments by the writer with other species of plants appear to confirm these findings. No change in reducing sugar content during exposure to ultra-violet radiation was found by Ranjan (243).

Reference should be made at this point also to the special metabolism of succulent plants. (For literature see 13, 84, 323a.) These plants exhibit a diurnal cycle of accumulation and disappearance of organic acids correlated with fluctuations of the respiratory quotient. During the night the acid content rises, while the R.Q. falls, sometimes to very low values; during the day the situation is reversed. This periodicity is correlated with the natural periods of darkness and light, and some investigators (*e.g.,* 251, 279), have ascribed the deacidification and concomitant carbon dioxide production primarily to a photolytic reaction. However, other factors, especially temperature, are also very important, so that the specific rôle of light seems to be far from clear as yet.

The availability of substrates for respiration conceivably could be determined also by the inter- and intra-cellular permeability. Numerous experimental observations have been interpreted as demonstrating that radiation increases the permeability of plant cells to a variety of substances (20, 36–38, 41, 42, 44, 130, 133, 138, 145, 146, 164-167, 170, 178, 190, 217, 234, 240, 257, 267, 290, 297-299, 311, 320, 321). Under other conditions radiation has been claimed to be without effect or even to cause a decreased permeability (31, 39, strate and (*b*) the rate of movement of materials in the plant or the exchange between plant and environment.

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Reference should be made at this point also to the special metabolism of succulent plants. (For literature see 13, 84, 323a.) These plants exhibit a diurnal cycle of accumulation and disappearance of organic acids correlated with fluctuations of the respiratory quotient. During the night the acid content rises, while the R.Q. falls, sometimes to very low values; during the day the situation is reversed. This periodicity is correlated with the natural periods of darkness and light, and some investigators (*e.g.*, 251, 279), have ascribed the deacidification and concomitant carbon dioxide production primarily to a photolytic reaction. However, other factors, especially temperature, are also very important, so that the specific rôle of light seems to be far from clear as yet.

The availability of substrates for respiration conceivably could be determined also by the inter- and intra-cellular permeability. Numerous experimental observations have been interpreted as demonstrating that radiation increases the permeability of plant cells to a variety of substances (20, 36–38, 41, 42, 44, 130, 133, 138, 145, 146, 164-167, 170, 178, 190, 217, 234, 240, 257, 267, 290, 297-299, 311, 320, 321). Under other conditions radiation has been claimed to be without effect or even to cause a decreased permeability (31, 39, 40, 131, 132, 147, 251, 257, 258, 304, 332). Unfortunately, the term permeability as used by various authors has doubtless included many distinct physiological phenomena. It seems not unlikely that the apparent influence of light on some of these is in reality the result of a respiration response rather than its cause.

The rate of transport of respiratory materials within the plant or within the cell also might be of importance in regulating the respiration rate. Protoplasmic streaming, or cyclosis, which no doubt plays a significant rôle in such transport, is intimately related to radiation. (For literature see 32.) In this connection it should be mentioned, however, that certain studies suggest that cyclosis may be controlled by respiration rather than the reverse (75, 287, 288, 296).

There exists, in addition, some evidence that growth-promoting substances, or auxins, may, under certain conditions at least, exert an effect upon plant respiration. Since the activity of auxin in the plant is known to be influenced by radiation, a conceivable mechanism might consist of the chain: radiation-auxin activity-respiration. Sufficient information to permit an evaluation of this possibility is not yet available.

Since respiration is commonly measured by gaseous exchange, it is obvious that factors which influence the rate of gas exchange even indirectly also affect the apparent respiration. For example, it has long been known that the gaseous exchange of many leaves is, to some extent, controlled by the degree of stomatal opening which is in turn closely dependent upon illumination conditions. Alterations in permeability or of other physical properties of protoplasm, such as viscosity, which might regulate diffusion rates, could play a rôle in this way too.

Other properties such as electrical charges and potentials of tissues, the pH of the cell sap and oxidation-reduction potentials of cell suspensions also are known to be influenced by radiation, on the one hand, and to be correlated with respiration processes, on the other. So little is as yet understood, however, of the cause and effect relationships which link these properties and processes that an extended discussion would not appear particularly profitable at the present time.

Finally, mention should be made of another phenomenon which appears to the writer potentially capable of playing a rôle in the observed effect of radiation on respiration in certain instances.

There is accumulating, from many types of experiments, evidence that certain plant cells possess a mechanism for storing carbon dioxide which can be released later (see *e.g.* 276, 277). If this release is influenced by radiation, as is indicated by other work (83), it might furnish an adequate explanation of some of the findings described above. Such a mechanism would appear to have, at most, only an indirect connection with the true respiration. (See also 171a.)

Emerson and Lewis, using the Warburg manometric technic, observed that illumination (by a sodium lamp at intensities slightly below the compensation point) of a suspension of *Chlorella pyrenoidosa* was followed by a large but short-lived effect on the rate of pressure change, which could be attributed to a production of carbon dioxide unaccompanied by a corresponding absorption of oxygen. The maximum rate of this carbon dioxide evolution, which under certain conditions amounted to several times the rate in the preceding dark period, was usually attained within a minute. The rate then fell rapidly to about the level of the dark value, and after passing through a much smaller second maximum exhibited a gradual decrease with time. On darkening the culture a reverse phenomenon resulted, namely, a transient uptake of carbon dioxide, of which the maximum rate was, however, much smaller than that of the evolution in light. Within a few minutes carbon dioxide began to be liberated, the rate increasing continuously over a period of several minutes. The rate of oxygen consumption exhibited only a small (perhaps insignificant) maximum, concurrent with the carbon dioxide burst in light and showed no anomaly when the suspension was darkened.

The amount of the extra carbon dioxide produced in light, beyond that equivalent to the oxygen consumed, was calculated to be of the same order of magnitude as the extra amount absorbed on darkening. This correspondence suggests the presence in the plant of some sort of reservoir of carbon dioxide which tends to empty itself on illumination and to become refilled in darkness. The effect was demonstrated to be connected with the vital activity of the organism, since boiled cells failed to show the response. Concentrations of phenylurethane which completely inhibited photosynthesis also suppressed the activity of the reservoir. In a concentration of 0.005% urethane, photosynthesis was reduced by 50%, but the initial carbon dioxide burst was unaffected, whereas the second slower phase of carbon dioxide production was completely inhibited. This finding suggests that the two phases of carbon dioxide evolution represent distinct processes. Franck has attempted to explain the phenomenon as a special case of the normal induction period of photosynthesis (90).

The characteristics of the carbon dioxide burst were found by Emerson and Lewis to depend upon a number of conditions. The amount of carbon dioxide evolved increased with the light intensity. Initial exposure to a low intensity only partially emptied the reservoir, and subsequent increase in intensity then produced a new burst of carbon dioxide. Maximal effects were obtained at intensities considerably below those required to saturate photosynthesis. Since the filling of the reservoir in darkness is a slow process (requiring several hours for completion), maximal light effects were obtained only after long dark periods. The amount of carbon dioxide absorbed by the reservoir is determined by the partial pressure of carbon dioxide in the environment. At carbon dioxide concentrations below about 0.5% only small bursts were noted in light. Large bursts were found at 5% carbon dioxide and still larger at 12%. The upper limit was not determined.

Both the filling and emptying of the reservoir are dependent upon temperature. At lower temperatures the bursts are smaller and less rapidly completed. The composition of the culture medium, especially with regard to the micronutrients, also was stated to exert an influence on the behavior of the reservoir.

SUMMARY

A review of the literature supports the conclusion that, under some conditions, an increase of the rate of "apparent" respiration, as measured by gaseous exchange, may be induced by irradiation of various species of plants and types of plant tissues. In the present elementary state of our knowledge it can not be decided with certainty whether or not the observed stimulations are directly related to the "true" respiration. Despite the long-continued interest in this problem the results thus far available are almost entirely of a descriptive nature, and in no single case has there been presented, as yet, a satisfactory elucidation of the mechanism involved. From a consideration of the diverse conditions and types of material with which an alteration of the gaseous exchange has been observed, it seems altogether likely, however, that such an effect may be the common end result produced by a variety of phenomena.

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HETEROSIS

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INTRODUCTION

Hybrid vigor, the manifest superiority of certain hybrids in size, yield and general vegetative vigor, has been recognized for at least two and a half centuries. The first artificially produced plant hybrids studied, those originating from crosses made by Kölreuter (80), furnished some excellent examples. Knight, the English horticulturist (79), noted the superiority of hybrids over pure types in many plants, and concluded that "nature intended that a sexual intercourse should take place between neighboring plants of the same species"—a conclusion which was to be elaborated in anoth half century by Darwin. Other very extensive work points to ea recognition of wide-spread occurrence of the phenomenon (48, 5 Mendel's famous paper (95) contains a statement reporting hybr of six to seven and one-half feet in height from crosses betw parents with heights of one foot and six feet, respectively. Jo (67) cites this case as an example of hybrid vigor, but the lusion may not be justified. Recent work tends to indicate eight is a poor measure of hybrid vigor in some plants. Da 27, 28) contributed many data to the problem. Parallelin ublication of Darwin's work, Beal (7) outlined a series of breed- g experiments in maize to make use of the superiority of crosse trains. The American Indians had, without modern scientifi nowledge, regularly planted mixtures of corn to increase the ield (23).

The observations of early investigators have been excellently sum- arized by Jones (67), while Collins (23) has discussed, in particu- r, the first work with maize. Reports prior to 1900 dealt mainly ith the occurrence of hybrid vigor, and there were few suggestions as to its causation. As Jones points out, it is to Darwin that credit must go for showing that hybrid vigor results from the union of different germinal complexes rather than from the mere act of crossing. Darwin approached the problem as no previous investigator had, through study of the effects of inbreeding. Since he was limited by working before the rediscovery of Mendel's work, he came to certain conclusions regarding the effects of inbreeding which we now know to be incorrect. Moreover, without knowledge of the Mendelian laws he was unable to recognize that inbreeding and cross-breeding are concerned with the same mechanism. Despite this limitation he saw clearly that hybrid vigor is the result of uniting organisms of differing constitutions.

THE RELATION OF HETEROZYGOSITY TO HYBRID VIGOR

It remained for G. H. Shull (117) to discern that the decrease in size accompanying inbreeding, and the increase sometimes obtained through hybridization are really different aspects of the same phenomenon. East (37) reached this same conclusion (122). In later papers (38, 41, 118, 119, 120, 121) these investigators were able to show clearly that what inbreeding does is to segregate into homo- gous lines the complex aggregation of genes found in normally ss-pollinating species. They demonstrated that inbred lines able of surviving will eventually become homozygous for all tors concerned. (The establishment of balanced lethals in cer- instances presents exceptions.) When two such homozygous s are crossed, the resulting hybrid commonly shows a marked ease in size and vigor, not only over its immediate inbred nts, but sometimes even over its more remote out-pollinating stors. East and Hayes (41) and G. H. Shull (121) concluded the stimulus to development possessed by the hybrid results directly from its heterozygosity. East and Hayes stated further that up to a limiting point, which they did not define, the degree of stimulation varies directly with the number of heterozygous factor pairs. In 1910 Bruce (13) had published a short paper proposing to prove a positive correlation between dominance and vigor. In the same year Keeble and Pellew (76) published an analysis of the inheritance of stature in peas. They concluded that the added height of the F_1 was simply the result of a combination of dominant factors for larger size. Height increases were one of the most often observed manifestations of hybrid vigor. Hence, might not hybrid vigor be explained on a similar basis? Here in the simplest terms are the two ideas around which most of the hypotheses dealing with the causal mechanism of hybrid vigor have been constructed—something inherent in the heterozygous condition on one hand, favorable action of dominant genes on the other. A third supposition, less easily definable in genetic terms, that hybrid vigor has its origin in some physiological stimulation, had been propounded before (72) and received some support about this time from A. F. Shull (116). It was at this juncture that G. H. Shull (121) proposed the term "heterosis" as a substitute for the awkward "stimulus of heterozygosity" and similar terms then in use. Shull went to considerable length to point out that more than direct gene action might be involved. He held that most of the stimulation is the result of gene action, but supposed that some of it could be derived from interaction between the male nucleus and the egg cytoplasm. Rather erroneously this term, "heterosis", has become established in the literature as a synonym for hybrid vigor. By the original definition "heterosis" refers to the developmental stimulation resulting, by whatever mechanism, from the union of different gametes. "Hybrid vigor" denotes the manifest effects of heterosis.

Before considering in detail the various proposed explanations of heterosis, it should be noted that the phenomenon is not at all confined to plants, although in them it is more easily studied and of greater realized importance. Certain early studied animal hybrids (10, 18, 53) are, however, clearly defined. Gerschler reported a cross between *Xiphophorus strigatus* (males 43.0 cm. long, females 52.0 cm.) and *Platypoecilius maculatus* (males 26.0 cm. and females 31.0 cm.) as giving "gigantic hybrids" (males 54.0 cm. and females 57.5 cm.). Bonhote obtained considerable size and fertility increases in *Meriones* crosses. Castle and Wright crossed domesticated and wild guinea pigs, obtaining hybrids whose body weights were considerably greater than those of either parent. Jones (67) has so adequately reviewed this early literature on hybrid vigor in both plants and animals that no further discussion is needed here.

THE "DOMINANCE OF LINKED GENES HYPOTHESIS"

Jones (66, 67) made what still stands as the most thorough study of the genetic aspects of the heterosis problem. He put f genetic interpretation based upon the presence of certain dominant linked genes. For the basis of his hypothesis Jones returned to the earlier work of Keeble and Pellew (76) noted above. These investigators had crossed two varieties of garden peas, each of which averaged five to six feet in height. The F_1 plants averaged seven to eight feet. The F_2 fell into four classes, closely approximating a 9:3:3:1 ratio. On the basis of this segregation, the writers assumed the operation of two genes, one producing thick stems, the other long internodes. Designating these factors as T and L, respectively, they assumed one parent to be of the constitution TT-ll and the other tt-LL. Both T and L were dominant to their allelomorphs. The F_1 was, therefore, taller than either parent because it contained both factors. Subsequent investigations have cast some doubt on both the validity of these data as explaining the genetic mechanism of height in peas, and on the wisdom of calling this case one of hybrid vigor. These facts, however, do not invalidate the very good example of the manner in which dominance may cause F_1 superiority over parental types. General acceptance of this dominance hypothesis as an explanation of heterosis had been precluded by two important objections. The first objection (41, 120) is that if hybrid vigor is due to the presence of a number of dominant genes having a favorable effect on development, certain individuals in the F_2 should be found to be as good as the F_1 because by the law of segregation they would be homozygous for the favorable dominant factors. Fixation of a pure breeding superior type would thus be possible. Actually F_2 segregates do not often even closely approach the F_1 in vigor. The second objection is that (43) if dominant genes are concerned in producing the favorable effect, no matter what their number is the F_2 should show an unsymmetrical distribution for the characters involved.

By the time Jones approached the problem, however, considerably more was known about the mechanism of heredity. Most important for the explanation of heterosis was Morgan's discovery of linkage (96). Jones seized the opportunity which linkage presented for explaining away the two objections to the dominance hypothesis. He developed his theory by supposing, as is now known to be true, that large numbers of genes are concerned with characters such as height, weight and size, which operate to produce hybrid vigor. Since there are large numbers of genes concerned, it probably follows that they will be located in several different chromosomes. The occurrence of many abnormal and deficient genetically-determined characters attests that not all genes are favorable to development. As Jones pointed out, it is safe to assume that no one variety or line has all the unfavorable factors nor has any single line all the favorable ones. Since most varieties of a given species fall within the same general range of size and degree of development, it is probable that each contains the same general proportion of favorable and unfavorable genes. If a majority of the more favorable genes exhibit some degree of dominance, even though it be small, then an explanation of the superiority of the F_1 is possible. The F_1 is the only generation whose individuals will possess the maximum number of different factors. Linkage makes it impossible for any individual of the F_2 to possess as many favorable factors as the F_1, and it will also explain the absence of the skewness that might otherwise be expected.

Actually both the absence of skewness and failure to recover an equally superior F_2 individual are explainable without the assumption of linkage. Collins (24) pointed out that with any large number of factors skewness would not show up in an ordinary sized population. Singleton (122) has calculated that two thousand times the total land area of the earth would be necessary to grow enough corn plants to stand an even chance of recovering a completely homozygous plant if 30 genes were concerned.

Jones' explanation has come to be called the "Dominance of Linked Factors Hypothesis". As East (40) said, it "was so probable that it was generally accepted, at least until 1930 (2), in spite of the fact that there was no direct proof for it". During the period from 1917 to 1930 there were many publications on the subject, but most of them merely record occurrences of the phenomenon.

THE PHYSIOLOGICAL MECHANISMS—SIZE AND RATE FACTORS

In 1930 Ashby (2) began publication of a series of papers on studies of the inheritance of physiological characters. In the first paper he set forth his purpose as being that of studying, by physiological investigations, the nature of the immediate cause of hybrid vigor. Using maize he was able to determine that for the period studied (20–70 days after germination) the hybrid had the same relative growth rate as its more vigorous parent. Further, it did not differ from either of its parents as to cell size, photosynthetic efficiency of the leaves, or time of flattening of its growth curve. The only hybrid advantages observed were higher percentage of germination and greater initial embryo weight. Ashby concluded that in this case at least the immediate cause of hybrid vigor was the larger hybrid embryo, since, as the hybrid growth rate was identical with that of the faster growing parent, this initial advantage was maintained throughout the grand period of growth. Murdoch (97) was later to demonstrate definite embryo size advantage in certain other maize hybrids. In a second set of experiments reciprocal hybrids of inbred maize lines were studied by Ashby (3). The results were essentially similar to those reported in the first paper. In each instance the hybrid appeared to have inherited the growth rate of the better parent as a simple Mendelian dominant. There was, however, considerable difference in the degree of vigor exhibited by the reciprocals. Ashby pointed out that this resulted from differences in embryo weight. The reciprocal seed weights differed, for although both were of the same genetic constitution, they had been matured on different maternal plants. The fact that embyro size is dependent upon other factors than genetic constitution may, he concluded, also explain some of the capriciousness of hybrid vigor. Ashby noted that Jones' hypothesis, based upon the action of favorable dominant genes, supposed superior metabolic efficiency on the part of the hybrids. Since his maize hybrids did not show any superiority over the parental lines by his measurements of growth rates, he came to the conclusion that some modification of the hypothesis was necessary. In the third paper (5), published in 1937, this subject is pursued further. Perhaps it is advisable to consider first some intervening papers.

Lindstrom (84) published the results of what he designated as a test of the Ashby hypothesis—the idea that hybrid vigor is due not to a greater efficiency index (relative growth rate) of the hybrid but merely to the maintenance of an initial size advantage. The test consisted of experimentally decapitating vigorous F_1 maize seedlings in early growth. Despite thus being handicapped the hybrids finally exceeded the parental strains as to dry weight of both plant and ear. This procedure did not, as Lindstrom supposed, reduce the embryonic capital in the sense in which Ashby intended the term (4). Ashby originally used the term to refer to the embryo itself, although later he restricted its use to the meristems within the embryo. Nevertheless, it did demonstrate effectively that Lindstrom's hybrids were capable of much greater growth per unit area of leaf than were the parental types. The discrepancies between Lindstrom's results and those of Ashby probably lie, as Lindstrom suggested, in the fact that he was dealing with the entire growth period, while Ashby was considering only a restricted portion of it.

In 1936 East (40) published a detailed critique of Ashby's work and made some further observations upon the heterosis problem. Taking first the matter of seed or embryo size, East pointed out that there are several recorded cases where hybrid seeds were not as large as those of the larger parent (39, 92). Certain of these cases are in the Leguminosae where there is virtually no endosperm, hence it would appear that embryo size alone can not be an important factor. East also adduced some corollary evidence from polyploidy. In *Triticum,* particularly, there are many tetraploids and hexaploids which show greatly increased vigor, although their seeds have much reduced endosperm and embryos no larger than those of their parents. Further, he called attention to a very pertinent fact in Jones' (67) data. Jones recorded seed weights, height at given time intervals, and final yields for maize crosses. His data indicate greater growth by the F_1's than by the parents, partly attributable to their advantage in seed size. The composite curve for the F_2's shows, however, that while these plants made their start from seeds larger than those from which the F_1's grew, they actually, in the final analysis, were smaller plants. If, as Ashby supposed, relative growth rate were controlled by a single gene, with the dominant gene producing the more rapid rate, such a condition would not be possible.

The work of some other investigators is of importance in consideration of this thesis. Miss Passmore (101) noted that in reciprocal crosses in the Cucurbitaceae the cross with the larger seed reached its mature size first, but that ultimately the smaller-seeded cross equalled it. She thus disclosed the importance of a point which Ashby did not consider—duration of the growth period. Collins (106) found much the same situation in maize. The large hybrid seeds showed a significantly higher growth rate for about two weeks and then no difference was detectable. From these data and others of his own East concluded: "(1) that seed size is a nutritional advantage, other things being equal; and (2) that it may be one of the manifestations of heterosis or may be the result of other causes, but that seed size, or the size of any part of the seed, can not be the true cause of heterosis." On the basis of these observations he took Ashby to task rather severely for proposing what he called the "Physiological theory of heterosis". This attitude was somewhat unfortunate, for, while at least one part of Ashby's results needed quite some qualification, Ashby recognized that he had succeeded only in pushing the problem back to the period between fertilization and maturation of the seed. He was concerned only with the immediate cause of hybrid vigor, and his evidence seemed to him indicative that, in his material, embryo size played an important part. The whole matter of embryo size in tomato has been considered in detail by Hatcher (60, 61) in papers which are a continuation of the Ashby series. Hatcher found that embryo size is dependent upon several factors, among them, truss position on the plant, number of fruit per truss, and most important, the number of fertilized ovules per fruit. He noted further that artificial pollination is much less effective than natural pollination. Since with artificial pollination fewer seeds are set, the hybrid seeds tend to be larger. He concluded that embryo size is of little importance in relation to heterosis.

Ashby's finding that the relative growth rate in his plants seemed to be determined by a single gene demands critical analysis. As Lindstrom (84) has pointed out, the existence of such a condition would be hard to understand. Ashby dealt only with a small part of the growth curve, the so-called "grand period of growth". This portion of the curve is generally supposed to represent the period of cell expansion, a supposition which may not always be entirely correct. It seems logical to assume that, other things being equal, the probability that certain environmental factors might act as "limiting factors" would be great during this period. In maize (141), at least, such seems to be the case. Inbred lines of the varieties Pipe and Pawnee and their reciprocal hybrids show markedly different reactions to varying light conditions. With some light intensities the hybrids show no superiority at all. Malinowski (92) noted that heterosis in beans produces responses similar to those obtained in certain environments. Such a condition would seem to suggest that in some crosses the hybrid will react differently than the parents to the limiting factor. Factors other than light are also known to be concerned. Rosenquist (113) found certain wheat hybrids to be very superior when spaced four inches apart but intermediate to the parental types when spaced at one-inch intervals. Until further evidence is produced it would seem better to assume that in certain crosses the hybrids inherit a single gene, or a complex of genes, which controls their reactions to some limiting factor or group of factors. Thus the hybrids might be expected to show the same general relative growth rate as one parent at least throughout part of the growth cycle without it being necessary to suppose so complex a character as the growth rate to be determined by a single gene. Further, it is doubtful that Ashby was justified in making statements concerning growth rate without having examined the early part of the curve. As will appear later many changes can and do take place in the growth rate during and immediately after seed germination.

The 1937 paper in Ashby's series (5) deals with hybrid vigor in the tomato. In this work Ashby considered the period from germination to the onset of flowering. His growth curves are thus somewhat more inclusive, although later work (139) has shown that his initial sampling interval of 10 days was too long. The results are essentially the same as previously reported. The hybrids showed vigor in having greater dry and wet weights, more leaves each of greater area, and a greater total assimilation rate (mg. CO_2 per dm^2 per day). Despite these advantages no hybrid superiority was detected as to relative growth rate measured as dry weight or height increase, rate of production of new nodes or leaves and expansion of total leaf area, cell size (upper epidermis of first and second leaves and hypocotyl), or photosynthetic rate (increase in dry weight per unit area). From these facts Ashby deduced that: (*a*) greater dry weight of the hybrids is due to maintenance of an initial advantage in embryo weight; (*b*) greater height is attributable partly to greater length of the primordium in the embryo and possibly partly to early more rapid elongation; (*c*) greater leaf number is due to the fact that the hybrid leaves begin to unfold earlier than those of the parents; (*d*) greater leaf area results from the fact that there are more hybrid leaves, each in a later stage of development than those of the parents; and (*e*) the greater total assimilation stems from possession of greater leaf area by the hybrid.

The most significant development here is that the original capital has been redefined as consisting of the primordia within the embryo rather than the embryo as a whole. This idea was developed further and the whole study extended in the fourth paper in the series (88). Luckwill's data are concerned mostly with the flowering period. They are in strict agreement with Ashby's previous findings. He does, however, give some figures purporting to prove that the shoot primordia of the hybrids were larger than those of the parents. Unfortunately the extent of these primordia is ill-defined and the figures seem to deal with promeristem, differentiated leaves and stem tissues as a unit. Luckwill himself admitted that they show but little, and it is to be doubted that they are valid even for purposes of comparison. Luckwill pointed out again that the findings of Ashby and himself necessitate some modification of the Jones' hypothesis of dominant linked genes. From their data it appeared necessary to assume that only one or at most only a few genes are concerned in the determination of relative growth rate (post germination rate). He then suggested two alternative mechanisms for production of heterosis in the hybrid embryos. If the genes which control pre-germination growth rate are the same as those which control post-germination growth, then embryo heterosis could be attributed only to a longer embryo growth period. This longer growth period might result from some initial stimulation—the old physiological stimulation theory of hybrid vigor. If the genes controlling growth rates are not the same, then some such mechanism as Jones proposed may be operative, but only in the pre-germination stages. Even had the observations of Ashby and Luckwill been complete enough to justify their conclusions, such a distinction as that outlined above would seem both unnecessary and improbable. Little enough is known about the developmental action of genes but the idea of independent gene action appears fallacious. It is not at all likely that the physiological processes of the post-germination period are entirely independent from and unconditioned by the physiological processes of the pre-germination period. Gene action is nothing akin to the static procedure of laying one unit upon another, but is a complex, highly integrated process in which each component is in some way related to every other one. One can accept Luckwill's first proposition and the "stimulus" hypothesis, but there is no evidence for it which can not be explained better on a genetic basis. But if one concedes that a mechanism such as that proposed by Jones is operative during development of the embryo, it would seem doubtful that dormancy, which does not even exist in some plants, would be a signal for surcease of this growth-controlling mechanism and inauguration of another. Even if this were so, the second would presumably be conditioned by the first in more than just embryo mass. As noted above, the answer seems to lie in Ashby's material in the presence of some single limiting factor operating in post-germination stages.

MANIFESTATIONS OF HETEROSIS

Heterosis is a real "double attraction". First, practical utilization of hybrid vigor is of immense value to agriculture. It has already increased the value of our corn crop by millions of dollars annually and its potentialities for other crops have hardly begun to be realized. Second, it presents ideal opportunities for the study of certain features of developmental genetics. Recent papers fall rather generally into two classes. One group has to do principally with the immediate causes and manifestations of hybrid vigor, the other with its genetic interpretation. It may be more profitable to discuss the former group first.

The prime requisite in any investigation is generally supposed to be an adequate definition of the phenomenon concerned. No such definition of hybrid vigor appears to exist. Kölreuter (80) spoke of greater size, increased number of flowers, and general vegetative vigor.

As Coffman (20) has pointed out, the effects of heterosis vary in different plants. Knight (79) noted increased height in pea crosses. Gärtner (52) recorded general vegetative luxuriance, increase in roots, height, number of flowers, and earlier and more extended period of bloom. With the advent of modern breeding methods more specific effects were recorded. For maize these are summed up by Jones (67). Among increases recorded are total yield, height, length of ear, number of nodes per plant, and number of grains per row. Root length, diameter and penetration are also greater in maize F_1's (78). Jones pointed out that the number of parts is affected much less than size of the parts. Investigators working with other plants, however, have recorded considerable increase in the number of parts, attended by little change in size. This is particularly true in tomato (88, 89, 139). The explanation of the differences necessitates recognition of the important fact that heterosis must be expected to exhibit different manifestations in plants of unlike growth forms. East (40) gave the best idea of the way in which heterosis is manifest when he said that invariably it is something which affects the organism as a whole. "Its effect is comparable to the effect on a plant of the addition of a balanced fertilizer to the soil, or to feeding a more adequate and more chemically complete diet to the animal". Maize is a plant with a determinate growth habit. Its single shoot meristem forms a limited number of leaves and then terminates as a floral inflorescence. The number of ears per plant is limited and usually constant within a strain. While it is true that improving the growing conditions of such a plant may lengthen the vegetative period and thus bring about some increase in the number of parts, such as the number of tillers, such action is somewhat limited. Added vigor goes then into increasing the magnitude of the parts already present. Within structural limits this increase is carried on from one part of the growth cycle to another. Larger leaves make for more photosynthesis, and larger root systems for more absorption. All these factors together make for greater yield—increased mainly in maize by larger kernels on larger ears.

In tomato the situation is different. Tomato is essentially an indeterminate plant. Whaley (139) has shown that heterosis here affects the size of the parts but little. Yield is increased by greater numbers of fruit rather than by larger individual fruit. In this typical "open" growth system added vigor goes into production of new organs, and the so-called "conservative" organs, the leaves, flowers and fruits, are little affected as to size. The same situation appears to exist in the soybean. Veatch (132) reported hybrid vigor in *Soja* as increasing node number, pod number, and seed number, but having relatively little effect on size.

The ultimate manifestations of heterosis appear, then, to differ somewhat with the nature of the plant. When individual cases are considered, with growth habits of the plants concerned in mind, it is apparent that the end effects are exactly what we should expect if the immediate cause of hybrid vigor were a raising of the efficiency of metabolism. They are the sort of results derived from a superior developmental system. Once superiority has been established its apparent degree may be considerably heightened, as G. H. Shull (121) has pointed out, by the fact that vigorous plants may be much less susceptible to the effects of varying environmental conditions.

It should be noted that investigators have used different bases for their estimations of superiority. The arithmetic mean or the geometric mean between the two parents for specific characters have been used in some instances. In other cases the hybrid was not considered to show added vigor unless it exceeded the better parent. Superiority over the better parent may be the more valid measure (21, 22, 132).

Whaley (139) made extensive experiments with hybrid vigor in tomatoes in an attempt to discover in detail some of the advantages possessed by the hybrids. Using, among others, one of the same crosses studied by Ashby and Luckwill, he found, as noted above, that heterosis produced its effects on total plant size. The determinate organs were subject to number rather than size increases. The findings supported those of Ashby as to relative growth rates during the grand period of growth, but marked differences were observed during the early post-embryonic stages and during the fruiting period. Hybrid embryo size advantage was found in one cross, but in another, showing even more hybrid vigor, the hybrid embryos were intermediate in size between those of the parents. In an attempt to test the validity of Ashby's hypothesis relating to the importance of the amount of "original meristematic capital", direct measurements were made of apical meristem volumes. Neither in the embryo nor during development was any relation found between volume of the meristem and development of hybrid vigor. In all the strains meristem volume was correlated with size of the determinate organs upon which heterosis had little if any effect. Bindloss (8) made similar observations on the size of maize meristems. However, it probably is to be expected that meristem size in the embryo will be conditioned by the growth habits of the plants. One might anticipate that generally maize and other highly differentiated embryos would show hybrid meristem size advantage more often than plants like tomato in which the embryo is much less extensively developed and in which heterosis has little effect upon organ size. Whaley extended his observations by studying cell and nuclear size changes in the apical meristem during development. Bindloss (8) had presented data on one cross which seemingly suggested that the heterotic hybrids possessed an advantage in nuclear size but no initial advantage in cell size. Kostoff and Arutiunova (81) had previously reported that large hybrids do not as a rule have larger root tip cells than their parents. Whaley found that in all cases development was accompanied by a progressive diminution in size of both cells and their nuclei in the apical meristem. The rate at which this diminution proceeded was, however, markedly less in the hybrids showing heterosis than in the inbred strains. Diminution also proceeded further in the hybrids than in the inbreds. The only other difference noted was in the behavior of the nuclei very early in development. In initial stages of growth the nucleus behaves differently from the cell in that for a short period either during or immediatedly after germination its volume increases. This increase is followed by progressive diminution. In the crosses studied the relative initial increase of nuclear volume was greater in the hybrids than in the inbreds. This behavior suggests differences in the physical biochemistry of the protoplasm, for this initial increase is due probably principally to intake of water. All differences observed indicate that far more than simple size characters are concerned. They rather suggest differences in metabolic level, in the efficiency of the processes of synthesis.

POSSIBLE GROWTH-SUBSTANCE RÔLES

Recent work of Robbins (108, 110) tends to support this view and to give indications as to what some of the factors may be. Robbins worked with fungi lacking the ability to synthesize certain growth substances essential for their own development. *Phycomyces Blakesleeanus* requires an external supply of thiamine or its intermediates for growth. When thiamine and extracts of partially germinated grains of maize were added to cultures of this fungus the cultures receiving extracts from hybrid grains showed greater growth than those to which extracts from the inbred parental grains were added. In the absence of thiamine the hybrid extracts did not have any greater effect than those from the inbreds. *Ashbya Gossypii* requires an external supply of biotin. When extracts from maize grains were added to cultures of *A. Gossypii* in the presence of biotin, the hybrid extracts again produced more growth than those from the inbreds. In the absence of biotin no differences in the amount of fungus growth were observed. Robbins stated that these facts seem to suggest the presence in the maize extracts of some growth substances other than thiamine or biotin. He called these unidentified substances factor Z, suggesting that Z is a growth-limiting factor which is synthesized in greater amounts by the hybrid than by the inbred lines. Robbins extended this investigation by using seeds of another cross. This time he also used extracts from dry grains. Although the results here differed somewhat, the hybrid extracts were again more effective in promoting growth than those of the inbred parents. To some extent this fact precludes the possibility that factor Z is the result of early growth rather than being active as a limiting factor. The possibility that it is a resultant rather than a causal factor during pre-germination growth still exists. These experiments give some indication that one of the advantages possessed by heterotic hybrids may be the ability to synthesize certain growth substances which the inbreds can synthesize less well if at all.

There is further evidence along another line which lends support to the growth substance idea. Robbins (109) and Whaley and Long (140) have shown that under certain conditions excised roots of hybrid tomatoes and maize grown in cultures show considerably more growth than those of their inbred parents. Robbins, using a cross of inbred lines of *Lycopersicon pimpinellifolium* and *L. esculentum,* found that in solutions supplemented by thiamine, thiamine and pyridoxine, or thiamine, pyridoxine and nicotinamide, the F_1 roots grew more rapidly and produced more dry matter than those of the inbred parents. Robbins was able to show that the roots of P_A showed a greater response to pyridoxine than those of P_B. The roots of P_B showed a greater response to nicotinamide than those of P_A. P_B approached the F_1 in solutions containing the three supplements. Assuming that the growth is limited in all of the lines by the ability to synthesize the three above mentioned substances, and probably a fourth unidentified factor which Robbins refers to White (142) as supposing may be glycine, the data indicate that the hybrid possesses greater relative ability to synthesize pyridoxine, nicotinamide and the unidentified substance. With pyridoxine and nicotinamide the results seem to suggest complementary factors from the parents combined in the hybrid.

Using a solution supplemented by thiamine, pyridoxine and nicotinamide, Whaley and Long (140) obtained essentially the same results for a heterotic cross between two inbred lines of *L. esculentum*. They were also able to report some significant results with maize, although the excised roots of maize are not particularly satisfactory in culture. In two different crosses early passage cultures (which may show some endosperm influence) showed considerable hybrid advantage. Growth reactions differed somewhat in the different solutions. In the absence of thiamine the hybrid advantage was very small. Growth was best in all strains when thiamine and nicotinamide were added. The addition of these two substances plus pyridoxine limited growth somewhat. Further work with the fungi such as that of Beadle and Tatum (6, 130) promises to yield valuable data concerning the genic control of some growth-promoting substances. It has been found that in *Neurospora* it is possible to induce mutations resulting in deficiencies for specific substances essential to proper growth. Strains with known deficiencies can then be combined.

OTHER PHYSIOLOGICAL FACTORS

Another suggestion of physiological differences appears in the work of Gregory and Crowther (57, 58) who found that some barley crosses inherit greater efficiency in the use of nutrient ions. Whaley's (139) work suggests a more rapid intake of nutrient materials by heterotic tomato hybrids. Others (19) noted that in maize the stomata of certain higher yielding types open earlier in the morning and remain open later in the afternoon than those of lower yielding inbred types. In addition, several investigators have made significant observations on the relation of growth rate to heterosis. Sprague (125) found that in maize the hybrids had a considerably faster growth rate during the early post-germination phases. Kempton and McLane (77) found that heterosis is often, but not necessarily, reflected in embryo size. They also produced evidence similar to Sprague's in finding that the rate of dry matter increase following dormancy in the hybrids was greater than that of the parents but that the advantage was not maintained to maturity.

SEED AND EMBRYO SIZE FACTORS

Any discussion of relation of growth rate to heterosis necessitates an understanding of seed size factors. The two major considerations are the control which the maternal parent effects over seed size and xenia and metaxenia. Miss Passmore's (101) results indicate the extent of the possible maternal control. This may come about through either nutritional factors or physical limitation by the size of the seed coat. It is important to remember that seed size is not necessarily any measure of embryo size—a point which led to some needless confusion concerning Ashby's data. Xenia and metaxenia have been noted by many investigators. Collins and Kempton (25) found hybrid seeds of maize to be 3% to 21% larger than inbred seeds on the same ear. Wolfe (143) obtained increases up to 16%. Jones (68) reported the same effect in other maize crosses. Paddick and Sprague (99) also reported large hybrid seed size increases. Despite seed size differences the reciprocal hybrid showed uniformity of size at maturity. Ware (134) has reported hybrid vigor in seed weight in cotton. Despite Ashby's contentions, there appears to be little evidence that an initial seed or embryo size advantage gives any lasting superiority. The initial advantage is usually lost before maturity is reached, not uncommonly in the first few days. The same condition seems to exist in certain tetraploids. Fabergé (45) reported a case in tomato where the tetraploid seed weight was 30% greater than that of the diploids. Eleven days after planting the size advantage of the tetraploids had disappeared. Fabergé emphasized that apparently early vigor in F_2 generations may have initial seed weight advantage as its sole immediate cause. Copeland (26) made an attempt to study embryo growth rates and found that some hybrid maize embryos showed added vigor at the 4–10 day stages. This fact suggests that mature embryo advantage is merely an expression of early developed hybrid vigor.

RECIPROCAL HYBRID DIFFERENCES

Whether some of the differences between reciprocal hybrids showing varying degrees of heterosis furnish evidence for cytoplasmic inheritance is still an unsolved problem. In *Lycopersicon* species crosses Schlösser (114) reported reciprocal differences in the osmotic value of the cell sap. His data seem to indicate strict maternal inheritance. Umeya (131) related a peculiar case in silkworms, *Bombyx mori*, showing the influence of nutritional effects. Eggs reared from transplanted ovaries were more vigorous than normal controls. The more inbreeding there had been prior to transplantation the greater was the effect in heightening vigor.

GENETIC BASIS OF HETEROSIS

Returning now to the genetic mechanism which results in bringing about hybrid vigor, it is desirable to consider further the "dominance of linked genes" hypothesis and the other possible explanations of heterosis. The Jones' hypothesis supposes the existence of large numbers of linked "size" genes. The term "size" gene is an unfortunate one. Size is merely the ultimate result of certain developmental processes. What the genes govern is the rate and magnitude of these developmental processes. That there are large numbers of them, linked in various groups, is certainly supported by all the evidence at hand. The hypothesis further supposes at least some degree of dominance of the "large size" genes, the genes producing the faster developmental rates or longer continued processes. On this point there is no experimental evidence. Such a supposition assumes either something in the nature of directional mutation or selection tending to eliminate the unfavorable dominants. The difficulty of detection of favorable mutations tends to obscure the whole matter. Further, the long cultivation, with attendant selection, of maize and other plants showing much hybrid vigor, makes the distinction between "favorable" and "unfavorable" factors a highly artificial one. Collins (24) pointed out this fact in his discussion of Jones' hypothesis. Collins was impressed by the deleterious effects of the many recessive mutations accumulated in maize. It is the action of these deleterious recessives that becomes apparent with inbreeding. Different varieties or strains may be expected to possess different assortments of these deleterious genes. In crossing, all such genes which are not common to both the parents are masked or suppressed by their dominant alleles. The hybrid is then more vigorous by virtue of the suppression of the depressing genes which are operative in the parents. This hypothesis requires fewer assumptions than does that of Jones, although it is not basically any different. It finds support in that the characters uncovered by inbreeding in maize are just such deleterious characters, most of them recessive, as would result in added vigor if suppressed. It does imply though, as does the dominance of linked genes hypothesis, that the majority of surviving recessive mutations must be deleterious, while the advantageous genes are dominant.

Dobzhansky and Rhoades (32) have proposed a scheme for detecting the presence of favorable and unfavorable genes in maize by the inversion method used for the detection of mutations in *Drosophila*. Their illustration for chromosome I explains the method. By X-ray treatment an inversion is produced in the chromosome which carries the dominant gene P (pericarp color). Homozygous individuals are obtained and crossed with lines or plants recessive for p. The F_1 plants are then either selfed or backcrossed to the homozygous p stock. Any excess of the Pp progeny over the pp ones will be due to favorable growth genes in chromosome I. By crossing the same inversion strain with different inbreds it should be possible to identify those lines having the most desirable complements of chromosome I genes.

Singleton (122) has raised the pertinent question as to whether, after one generation of backcrossing, the differences between the lines compared would be large enough to be revealed, or whether they might not be masked by the heterozygous constitution of the other nine chromosomes. He suggests use of the multiple recessive method for detecting favorable factors.

East (40) proposed a new interpretation of the genetic mechanism, partly in order to overcome the objections to the assumption of dominance of more favorable factors which is essential to the Jones' hypothesis. Citing crosses between "purged" inbreds as showing heterosis, East concluded that although the diminution of vigor accompanying inbreeding is due to the unmasking of deleterious recessives, these genes have little to do with heterosis. It is difficult to see how it would be possible to "purge" inbred stocks. Many of the deleterious genes involved must be ones whose effects are to produce degrees of defectiveness so small as not to be readily detectable. Some degree of hybrid vigor might still result from crossing of the so-called "purged lines" and be due to suppression of recessives. Jones (71) has pointed out that there are many deficient loci in maize after long inbreeding has eliminated all the visible defectives—those in which the deleterious action is readily measured. East then proposed a division of genes into two classes—normal and defective alleles. Since for the most part these combinations of normal and defective genes, as N and n, show complete dominance of the normal and the effect of NN is thus not measurably different from that of Nn, these combinations can not be of importance in producing hybrid vigor. He concluded that the key to heterosis is to be found in the behavior of normal genes which control the inheritance of quantitative characters. East repeatedly drew division lines between classes of genes, setting off the qualitative genes from the quantitative ones and those affecting morphological pattern from physiological pattern. This arbitrary distinction is useful in some respects but it creates considerable confusion and obscures his explanation of the heterosis mechanism. He assumed that there are great numbers of different alleles among the normal or nondefective genes, the group which primarily controls the physiological efficiency of the organism, the set of characters related to heterosis. All evidence to date indicates an absence of any considerable degree of dominance in so-called quantitative character genes and suggests rather that each member of an allelic pair exerts an effect upon the ultimate character expression. East suggested, therefore, an hypothesis which combines part of Jones' linked gene theory, part of Rasmusson's (104) quantitative factor hypothesis, and the old idea that heterozygosity is in some way concerned in heterosis. His idea may be stated briefly as follows: size traits are controlled by a large number of genes in various linkage groups; among these genes dominance is virtually nonexistent but there are numerous multiple allelic series; if in a given series each member affects a different physiological condition, then the heterozygous condition may be expected to produce cumulative results; that is, if a_1 affects a somewhat different process than its allele a_2, a_1a_2 may have a greater effect than a_1a_1 or a_2a_2. This hypothesis implies some sort of complementary action between alleles—a_1 supplies what is lacking in a_2, or *vice versa*. East did get away from the dominance issue, an advance, since, as pointed out above, there is little good evidence for even partial dominance of larger size genes or more favorable growth genes. However, the essential assumption that the mutated alleles of a series affect different processes which in combination produce some kind of complementary or cumulative effect, is without much evidence to support it.

Jones (69) did find possibly advantageous allelic differences from parental types in heterozygotes of *Nicotiana*. Dunn (36) has reported a case of lethal alleles in mice acting in such a way as to permit viability in the heterozygotes. Alleles, with such an additive effect as East supposed, have been found in *Godetia* (62).

However, there is not much ground for suggesting that these interactions play any considerable rôle in heterosis.

Significant contributions to the theory of the genetic mechanism responsible for heterosis appear in recent work by Dobzhansky and Sturtevant and their co-workers (30, 31, 33, 128, 129), which is not primarily concerned with the heterosis problem. Working principally with *Drosophila pseudoöbscura,* these investigators have shown that in natural populations there are large numbers of more or less unfavorable recessive mutations. Cross fertilization permits accumulation of these up to an equilibrium point which is determined by the mutation rate and the population structure. Where the population size is of sufficient magnitude there exists a great variety of chromosomes with a diversity of gene content. In such populations cross-bred individuals greatly exceed the homozygotes in vigor by virtue of suppression of unfavorable recessives. Jones (71) has clearly recognized the importance of this type of gene action in producing vigorous hybrids. An explanation of some hybrid vigor on the basis of such gene action rests upon the same fundamental principle as that of the original hypothesis of G. H. Shull and Jones, but it does permit a somewhat different interpretation of the phenomenon.

If heterosis is due to the suppression of unfavorable genes and not to some special superiority on the part of the dominants, a change in the conception of what heterosis constitutes is necessary. The literature is pervaded by the idea of hybrid vigor as something better than the "normal", if the term is permissible. The hybrid is thought to have an absolute advantage. This view has always seemed curious when one considers what happens during continued inbreeding in a plant like maize. If the suppression hypothesis is accepted it is to say, in effect, that the hybrid represents the "normal" or biological optimum under a given set of conditions and that the parents, whether purposely inbred or not, are inferior types—they contain an accumulation of deleterious factors. Such an idea certainly fits the facts of maize breeding.

An examination of this hypothesis in the light of what is known about heterosis adds weight to its validity. Arbitrarily the deleterious genes may be arranged in a descending scale of effectiveness as: lethals → semi-lethals → powerful developmental modifiers → weak developmental modifiers. Dobzhansky has emphasized the existence of all these classes of factors in his material, and the general body of evidence points to their presence in all mutating organisms. Since their effect is one of complete elimination, lethals are of no interest in relation to heterosis. The other factors, and particularly their degree of effectiveness, are, however, important.

Plants may be divided into three groups—those which are normally completely cross-pollinated, those completely self-pollinated, and those in which both cross- and self-pollination take place. In the cross-pollinated types, except under certain conditions, an accumulation of semi-lethals and both the powerful and weak developmental modifiers may be expected. This accumulation is conditioned by the mutation rate and by the size of the effective breeding population. A low mutation rate would limit the number of deleterious factors accumulated, but time must also be taken into consideration. Effective population size is an equally limiting factor. If the breeding population is so small as to permit frequent union of gametes carrying the same deleterious factors, then the rate of elimination will be high. Any considerable accumulation of these factors can take place only in populations large enough to contain many different chromosomes and not have them eliminated by frequent *like* gametic combinations.

In self-pollinated plants the effective population size has been reduced to the minimum. Immediate elimination of any mutation with a considerable detrimental effect is a certainty. Only those mutations whose detrimental effect is weak enough to allow them to persist without being suppressed by normal alleles can accumulate. These plants can accumulate, then, only those mutations which fall in the class of weak developmental modifiers. And these can accumulate only to the point where their combined effects begin to produce elimination. In plants where self-pollination is not obligate, the population size is somewhat larger. Its exact size is determined in part by the relative proportion of self- and cross-pollination. Here if the population size is large enough, the more powerful of the developmental modifiers may also accumulate. In very large effective populations even a few semi-lethals might be found.

Any actual measure of comparative amounts of heterosis is, of course, not possible. However, it is of considerable interest to obtain a general idea not only of the degree of added vigor and the consistency with which the phenomenon occurs in any given organism, but also in what type of organisms it is most frequently encountered. Engledow and Pal (44) made a survey of what they considered well authenticated instances of hybrid vigor and came to the conclusion that it occurs most often in crosses between plants which are diploid, normally cross-pollinated and endospermic. Reliable numerical data are few, and no standards of comparison exist, but in the general body of literature the following facts stand out. Hybrid vigor is exhibited by organisms of all classes. It is found in *Lycopersicon, Phaseolus* and *Soja* where obligate self-pollination is approached; the cereal grains, which range from highly self-fertile, completely self-pollinating forms, as in certain ryes, to normally more or less completely out-crossed forms like maize. Completely self-sterile plants as certain nicotianas show it when crossed. It has been pointed out several times by various investigators, notably East (40), that within certain limits the amount of heterosis varies directly with the genetic disparity of the stocks. Unrelated lines show more heterosis than related ones. Karper and Quinby (75) have even attempted to use the amount of hybrid vigor as an index of the degree of relationship between parents. Inbreeding prior to crossing tends to greatly increase the amount of heterosis in heterogamous plants. The limit to the increase is the point where, as East (40) has expressed it, "the genic differences fail to nick". To a certain extent it follows that the amount of heterosis will increase as obligate cross-pollination is approached, since the basis of greater genetic disparity is laid more rapidly in an out-pollinating population if it is not too small. Controlled inbreeding heightens this disparity.

For example, in maize, particularly in purposely inbred lines, the yield of the hybrids is often many times greater than that of the inbred parents and may exceed that of the original heterozygous individuals by more than 50%. Further, the relative number of crosses showing hybrid vigor is great. In tomato, on the other hand, the hybrid advantage in yield rarely reaches 50% (Alabouvette and Titard (1) give a range of 25% to 40%), and heterosis occurs only once in a number of crosses. It would appear, then, that heterosis is more prevalent and hybrid vigor greater in those forms whose method of reproduction permits accumulation of the more deleterious mutations and, it might be added, where selection has heightened this accumulation. Heterosis is less outstanding and a much more elusive phenomenon in those forms whose method of reproduction eliminates all except the less deleterious genes.

Heterosis in maize is worth further consideration both as maize is the type of organism in which hybrid vigor is most manifest and because it is the best known and so far most economically valuable case. Fisher's (46) genetical theory of natural selection has a direct bearing upon the possible explanation of heterosis in organisms like maize. Fisher maintained that in a population where heterozygous individuals predominate, the heterozygous condition will be optimum for many pairs of genes. Concerning population size in maize we have no experimental evidence. However, maize is wind-pollinated and grown in cultivation over large areas. These two facts indicate an effective population size much greater than that of self-pollinated or specific insect-pollinated plants. The very nature of progressively inbred lines of maize is certainly evidence enough that inbreeding uncovers large numbers of deleterious recessive genes. There is proof, too, that these genes range from slight developmental modifiers to lethals in the fact that generations of inbreeding usually produce strains weakened to various degrees.

Jones (70) has recorded the results of a long continued inbreeding program in maize. Of four original lines, one line and one second generation separated sub-line failed to survive. The surviving lines were selfed for 30 generations. Reductions in height were encountered in each of the first five generations, yield reductions in the first 20 generations. Jones' data also give evidence of continuing mutation, since sub-lines separated at different times showed transmissible variations in some instances and not in others, Jones has noted that none of the variations which appeared during the whole period of inbreeding was such as could be interpreted as favorable to survival. It must be borne in mind, however, that, where hybrid vigor is concerned, it is the expression of the character in the hybrid rather than in the inbred which is important. Singleton (122) and others have stressed that the only way to test the merit of an inbred is by crossing.

Supposing it were possible to start with two maize plants which were at least to a certain degree homozygous, crossing these plants would produce a heterozygous F_1:

$$\begin{array}{ccc} \text{AAbbCCdd} & \times & \text{aaBBccDD} \\ & \text{AaBbCcDd} & \end{array}$$

If the recessives were deleterious even to a limited degree, then the hybrid would be superior to either of the parental types. Any heterozygous individual would be superior to a homozygous individual, since if there were any considerable number of factors involved, an individual approaching homozygosity would possess certain of the less favorable recessives. Superiority in any number of traits or characters would most likely include some which give a selective advantage.

Fisher (47) has made the suggestion that in certain cases there is an avoidance of homozygous dominants. He calculated that in *Paratettix texanus* some 40% of them were eliminated in each generation, supposedly because of an accumulation of unfavorable factors made possible by some selective advantage favoring the heterozygotes. In maize certain dominant mutations, such as Rg, ragged leaf (12), and Tp, teopod (83), are known to have much more deleterious effects in the homozygous than in the heterozygous condition. It may be supposed that other genes with less obvious effects might operate in the same manner.

The way in which maize culture has been handled, even by the Indians, would seem to indicate that, consciously or not, there has long been a process of selection favoring the heterozygote. Thus it is perhaps possible that some of the heterosis in maize may result directly from heterozygosity rather than from the action of dominant genes either in the sense of "more-favorable" factors or as recessive suppressors. With these genes, then, the distinction between recessives and dominants is non-existent or unimportant. It ought to be pointed out that this possible importance of heterozygosity is not based upon the supposition of an initial germinal stimulation but upon a specific type of allelic interaction. The mechanism is similar to that proposed by East (40). It is not, however, based on complementary action of alleles affecting different processes, but upon the assumption of greater advantage, perhaps physiological efficiency, for the heterozygote, arising from the fact that certain alleles may have less of a weakening or other unfavorable effect when heterozygous than when homozygous.

Heterozygosity rather than dominance has always been a rather appealing possibility for explaining maize heterosis. Stubbe and Pirschle (127) cited the behavior of a certain mutant race of *Antirrhinum majus* as evidence in the support of the heterozygosity theory. They obtained a race with a chlorophyll deficiency and a tendency to mutate back to the normal in both the somatic and generative tissues. Upon selfing, variable numbers of phenotypically normal individuals were obtained. These individuals were heterozygous with respect to the mutant gene. They exhibited a distance degree of hybrid vigor. The authors maintain that this case constitutes proof that heterosis is due to heterozygosity rather than the additive effects of dominant factors.

On the other hand, other suggestions presented lead away from the heterozygosity idea (105, 107). These investigators have developed the so-called "convergent improvement" system in maize. In this work the F_1 hybrid is backcrossed to each parent through several generations, while selecting in each generation for the favorable characters of the non-recurrent parent. The inconclusive data suggest that hybrids between partly recovered lines may be more vigorous than those between the original parents, even though they are less heterozygous. Murphy (98) has more recently secured hybrids from converged lines which yielded more than the original F_1 cross. This suggests incomplete dominance of the greater yield factors in the F_1.

Singleton (123) has reported what he interprets as a single factor for heterosis. Purdue 39, a widely used sweet corn, when inbred mutated to a dwarf form, C_{30}. The progeny from crosses between P_{39} and C_{30} indicated that a single recessive mutation was involved. Crosses of the mutated form with other inbreds, C_{13} and C_{15}, sometimes produced hybrids giving greater yields than crosses between P_{39}, the normal form, and these same inbreds.

Powers (103B) has recently proposed what he terms an expansion of Jones' theory for the explanation of heterosis. From earlier investigations (103A) Powers concluded that actually heterosis and dominance are expressions of the same genetic phenomenon. Applying the term "heterosis" to a favorable end result such as yield under a given set of conditions he was able to show that although the multiplicative effect of a given pair or set of genes results in heterosis, some of the individual genes may show dominance for an unfavorable character, such as smaller fruit size. He points out that there thus may be no dominance, or partial dominance of "unfavorable" genes and still be heterosis for a given character if the gene action is multiplicative. By his association of heterosis and dominance Powers emphasizes a point of importance, for if the two are degrees of expression of the same physiological mechanism, then, as there is dominance of both favorable and unfavorable alleles there must be a condition of "unfavorableness" balancing what Powers has termed "heterosis" and for which this paper has used the less scientific term "hybrid vigor." Although little studied this condition has been recognized by several investigators and is generally referred to as "negative heterosis." Powers proposed "non-beneficial heterosis," a much better term, since in discussing the phenomenon we are limited, so far at least, to a consideration of end results beneficial in an agricultural or horticultural sense rather than in relation to natural conditions.

Mather (94) has made some interesting suggestions concerning heterosis in a recent review paper. He points out that survival under natural selection hinges upon proper genetic balance within the organism. In homozygous inbreeding organisms this balance is mainly an internal one—among individual gene combinations. In outbreeding organisms, such as maize, this internal balance is over-shadowed in importance by relational balance among pairs of chromosomes working together. Intercrossing brings together different polygenic combinations. If these combinations have not been selected for good relational balance, the hybrid will show a greater or lesser departure from the optimum for the conditions under which the organism exists. Hybrid vigor is the designation given to such a departure when it is in the direction of increased size. It follows that since hybrid vigor is a sign of poor adaptation it will be selectively disadvantageous under natural conditions.

Mather highlights the already apparent relation between artificially imposed conditions and the occurrence of hybrid vigor. He points out that heterosis is the automatic consequence of diminution in the freedom of interbreeding among strains, and an effective agent in the development of isolating mechanisms and hybrid sterility.

A few cases which may actually be produced by factors other than heterosis deserve mention. In beans (91), soybeans (132) and rye (11) there have been instances in which hybrid vigor appeared to be carried beyond the F_1 generation, even into the F_3 and F_4 in the beans. It remains for such cases to arise under very carefully controlled conditions before any detailed consideration is necessary. Some minor cultural variation from one year to another might easily give rise to the condition. It is probable that cases appear in which the homozygous condition for some factors is superior to the heterozygous or in which large numbers of the advantageous genes are grouped in individual F_2 and F_3 segregates.

POLYPLOIDY AND HETEROSIS

The significance of polploidy in relation to heterosis has been studied but little. Huskins and Smith (65), who recognized the implications of Fisher's suggestion (46) that under certain conditions the heterozygous state may be superior to the homozygous one, have discussed the matter briefly. They supposed a specific type of hybrid vigor to result from certain combinations of alleles in all cases. They assumed that in an obligate cross-fertilizing organism there is added to this specific action a more generalized one dependent upon small differences between many alleles. An autogamous allopolyploid should exhibit the specific type of hybrid vigor of its diploid ancestor. An allogamous polyploid should have this specific type of hybrid vigor plus the generalized type. It would seem difficult to separate the two types of hybrid vigor. Degree of the differences will be dependent upon the number and nature of the accumulated mutations. The apparent specificity of the phenomenon in autogamous organisms is conditioned by the fact that a certain degree of dominance is operative. In the allogamous organism, if, as has been supposed for at least some cases, the heterozygous state is superior, dominance and recessiveness have disappeared, their absence makes the detection of specific differences difficult—hence perhaps the appearance of a generalized effect.

East (40) came to the conclusion that natural polyploids do not show hybrid vigor. This would mean either that amphidiploids do not possess heterosis or that natural polyploids do not originate from hybrids. Artificial polyploids, according to East, often do show hybrid vigor. He cited the case of *Spartina Townsendii* which is an amphidiploid from a cross between *S. [illegible]rnifolia* and *S. stricta.* So vigorous is the polyploid that it has replaced the parental forms (64). Other such forms are *Primula kewensis,* a turnip × swede cross (51) and *Nicotiana glutinosa* × *N. tomentosa* (54, 42).

A word should be said about hybrid vigor in organisms other than the green plants. Dickson (29) reported what he termed "hybrid vigor" in one cross between strains of *Neurospora crassa*. Since the mycelial growth is haploid the term is hardly applicable. However, Dodge (34) has reported in *Neurospora* a very interesting phenomenon which is perhaps related to heterosis. He combined strains of *N. tetrasperma* in which nuclear migrations occurred so that nuclei of both strains came together in a common heterocaryotic mycelium. In certain such "crosses" the heterocaryotic mycelium grows much more rapidly and abundantly than that of either component. Dodge termed this phenomenon "heterocaryotic vigor" as distinguished from "segregant haploid vigor" (as reported by Dickson) and from the true "hybrid vigor" of diploids.

HYBRID VIGOR IN ANIMALS

Earlier data on hybrid vigor in animals have already been mentioned. Some consideration of later recorded cases is warranted to show the universality of the phenomenon. Generally speaking, however, hybrid vigor has been less studied in animals than in plants. It has been reported in most of the laboratory animals which have been studied genetically. Marshak (93) found it in mice—with considerable difference in degree in reciprocals. He attributes the reciprocal differences to genome-cytoplasm reactions. Little and Tyzzer (86) had earlier reported heterotic F_1 mice in which tumors grew at a more rapid rate than in sib-crossed lines. Castle (14) interpreted this as being due to the heterozygosity of the F_1 mice. Grüneberg (59) recorded what he believed to be the largest recorded litter of mice—19, with an average birth weight of 1.54 gms.—in hybrid mice. Green (55, 56) had reported several cases where hybridization increased litter size. Fortuyn (49, 50) obtained like results. Strong (126) made an observation similar to that of Little and Tyzzer by noting that transplanted tumor tissue grew about 25% faster in heterozygous F_1 mice than in the parental homozygous inbreds or the F_2 or F_3 individuals. Vicari (133) suggested that the faster maze learning exhibited by F_1 hybrids between *Mus musculus* and *M. Wagneri* might be due to heterosis. Whether heterosis is actually active in instances such as these can not really be determined until we know more about specific genic effects. Livesay (87) found added vigor of varying amounts in different rat crosses. In hybrid vigor occurring in crosses between guinea pigs and *Cavia Cutleri,* Castle (16) drew an important distinction between operation of specific size factors, which in this case show incomplete dominance, and heterosis. Wright (144, 145) also observed heterosis in guinea pig crosses. Numerous observations are recorded relating to the presence or absence of hybrid vigor in rabbits (15, 17, 100, 103). There are several cases among animals of economic importance. Roberts and Laible (111) reported a case in swine which has been interpreted many times as showing the establishment of early size differences resulting from heterosis. They double mated a Duroc Jersey sow first to a Poland China, and then to a Duroc Jersey boar. At birth the cross bred pigs were significantly larger than the pure breds. At six months the crossbreds were much heavier. Whether this is really a case of hybrid vigor is not clear. Others (115) made the reciprocal of this cross, mating a Poland China sow first with a Poland China and then a Duroc Jersey boar. They found the inbreds to be heavier at birth, but the hybrids gained weight more rapidly after birth. Still others (147) have noted sheep crosses of Merinos × Hampshiredown which gave progeny of higher than the purebred birth weight, and faster post-natal growth. Cattle-yak hybrids have been subject to several investigations. Lus (90) reported F_1 hybrids heavier and generally larger than the parents. Kushner (82) found that such hybrids greatly exceeded their parents in the blood characters of Hb content, number of erythrocytes, and alkalinity. This condition makes for greater oxidizing power which in turn produces more intense metabolism and more rapid growth. Kushner reports similar findings in hybrids between dromedaries and bactrians, and in the mule, which was accepted by all the early writers as the classic animal example of hybrid vigor. Heterosis has been noted also in Zebu × Yak crosses (146).

In fowl hybrid vigor has been reported in many cases. Dunn (35) noted the greatest manifestations as being the hastening of sexual maturity. Jull (73, 74) found hatchability and vigor to be increased by crossing. Others (137, 138, 148) have pointed out that hybrid vigor occurs in chicken crosses only in cases where the parent stocks are very homozygous. Warren (135, 136) crossed Jersey Black Giants and White Leghorns and found that the F_1 hybrids were better egg producers, had lower embryonic and chick mortality, and grew faster. It is of interest that none of the reports of heterotic chicken crosses indicates that the hybrids are more than intermediate to their parents as to mature size.

Among insects hybrid vigor has been reported in the silk worm, *Sericaria mori* L. (9). Pearl, Parker and Gonzalez (102) proposed that increased life span in certain *Drosophila* crosses may be a manifestation of hybrid vigor. Robertson (112) suggested the possibility that hybrid vigor is concerned in parthenogenesis in the Tettigidae. He supposed that heterosis might possibly stimulate cleavage in the egg early, thus beginning the development of an embryo.

To the case in fishes mentioned above can be added certain intergeneric sunfish crosses (63). Hybrids of *Apomatis cyanellus* × *Eupomatis gibbosus* greatly exceeded either parent in size and weight.

CONCLUSION

Any attempt to arrive at a single definitive explanation of the genetic basis for the phenomenon of hybrid vigor seems unwarranted with the evidence at hand. As Lindstrom (85) has pointed out, there are no critical proofs for any of the so-called "heterosis theories". Some hybrid advantage is undoubtedly due to simple factor combinations—complementary gene action as usually understood. Much of it is due to the suppression of deleterious recessives in the hybrid, or the favorable action of dominants. The interpretation of the concept in this case turns on the degree of deleteriousness assigned to the recessives. Stated either way it means that the dominant genes concerned are relatively more advantageous to the organism than their recessive alleles. Heterozygosity of certain alleles may account for some of the hybrid superiority where mutation and selection have brought about a degree of advantage associated with the heterozygous condition. Any or all of these operating mechanisms can bring about hybrid vigor in the progeny of a cross.

The manifestations of heterosis depend upon the factors involved, and if elevation of the general metabolic rate is concerned, on the growth form and characteristics of the organism. The fundamental processes responsible for the production of hybrid vigor may differ somewhat in various organisms, but all of them are factors which increase the physiological efficiency of the hybrid organism. The end effect is a general one, although the immediate effect may be as specific as the slight increase of a given substance at one stage in development.

Further, it should be kept in mind that hybrid vigor is a phenomenon observed and studied, for the most part, in organisms under domestication. Like other effects its level may be supposed to differ in response to changes in environment.

As Smith (124) has stated in an excellent recent review of quantitative inheritance, solution of the heterosis problem awaits the presentation of much more evidence concerning the general action of genes governing size.

Much knowledge regarding the phenomenon of heterosis should be derived from the present day genetic studies aimed at investigating gene action in controlling physiological reactions and their interrelations in various organisms under carefully controlled conditions. In problems of developmental genetics the background of hybrid vigor offers a more fertile field for study than ever before.

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SPORES AND POLLEN AS MICROFOSSILS

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INTRODUCTION

Spores and pollen have long been known as fossils, but it was not until approximately thirty years ago, when statistical methods were applied to the fossil pollen of European peats, that a wide interest became apparent. By statistical methods it is possible to determine much concerning the development of earlier vegetation, and from that to derive other information of scientific and cultural value. Interest in the older fossils has been rekindled, but as yet these have not received the attention given the pollen fossils of Quaternary age.

The term "plant microfossil" is used here for spores and pollen grains of plants irrespective of their affinity. Algae, fungal hyphae, trichomes, certain leaves and other plant parts that might come under the heading of microfossils are not included in the present discussion.

The literature pertinent to plant micropaleontology is widely scattered through periodicals of many sciences. It is to be found not only in publications of botany and geology, but also in those of zoology, chemistry, physics, and those of economic interest.

OBJECTIVES OF THE STUDY OF PLANT MICROFOSSILS

There are three major objectives in the study of plant microfossils: (*a*) to establish the biological affinities of isolated fossil spores and pollen, and to determine their phylogenetic relationships; (*b*) to use spores and pollen as indices in the stratigraphic study of rocks; and (*c*) to aid in the understanding of paleogeographic and paleoecologic problems.

(*a*) There are thousands of unassigned species of spores and pollen existing as isolated bodies in shales, coals, clays and peats. It is not conceivable that all spores and pollen ever will be assigned to their natural species, since there are similar forms in widely divergent groups, and also, as in other biological phenomena, similarity of form increases with genetic proximity. Very frequently in modern plants, species within a genus are not distinguishable from each other on spore or pollen characters, and it can be safely assumed that this is also true for ancient plants. Certain extinct species of Tertiary plants have spores and pollen which closely resemble existing species. Such a condition complicates micropaleontological problems, but some of these may be solved with the completion of extensive morphological studies. When these investigations are well under way, it may be that a combination of micro- and macrofossils will be used in the delimitation of various genera or species. In the modern heaths, for instance, it is difficult to separate certain genera on the basis of pollen, but because the leaf trichomes are quite diagnostic, it is frequently a simple matter to determine the identity of some of the fossil heath pollen that are encountered in postglacial sediments. In the older rocks it may be possible in the future to distinguish certain species of *Lepidodendron* by a combination of leaf and spore fossils.

In the pursuance of fossil morphological and evolutionary studies, it will be necessary to learn a great deal more about modern spore and pollen morphology. Though numerous studies of fossil fructifications have been made, they represent only a beginning of a very necessary part of the chain of knowledge leading to the maximum usefulness of microfossils. To illustrate the type of studies needed, papers on the structure of *Rhynia* (35), on the morphology of the pollen in *Cordaites* (23) and on *Senftenbergia* (62) may be mentioned.

(*b*) The present use of plant microfossils in stratigraphy lags far behind the use of animal microfossils, though both were discovered at approximately the same time. Several reasons account for the difference, but the most important appears to be the lack of knowledge concerning the identity of the plant fossils. Such knowledge is now being pursued, and the results already attained from the late Paleozoic, Mesozoic and Cenozoic rocks testify to the stratigraphic value of plant microfossils, and indicate the development of a useful new adjunct to this field of geology.

Animal microfossils, such as the Foraminifera, sponge spicules and ostracods, occur widely through the limestones, shales, fine sandstones and their clastic equivalents. They do not occur very frequently in coals or peats. On the other hand, plant microfossils occur most abundantly in coals and peats, less frequently in limestones, shales and fine sandstones. Therefore, it would seem that together the two would complement each other in a thorough study of sedimentary rocks.

In pre-Quaternary rocks the emphasis of plant micropaleontology has been placed upon stratigraphic value, an interest that has not been fully explored by workers in the clastic sediments of the Quaternary period. In the latter, the time intervals have been relatively short, and we still lack a complete understanding of the glacial drift pattern. These factors, coupled with the problems of forest succession and probable climatic shifts, presents variables that will require years of intense research to comprehend.

(*c*) The extent to which plant microfossils have been used in determining the paleogeography and paleoecology of plants diminishes rapidly with the increased age of the rocks and decreased knowledge of plants. The science of pollen-statistics, as known to most people, has dealt mainly with glacial and postglacial sediments. Great emphasis has been placed upon the determining of forest succession, climatic shifts and plant geography. Results from these studies have reached rather more definite conclusions in Europe than elsewhere.

Attempts to discuss geographic and ecological problems in pre-Quaternary microfossil deposits are exceedingly few. Wodehouse (95) and Simpson (75) have described in some detail the plant ecology of the Eocene from deposits of oil shale and coal. Back beyond the Tertiary the problems of paleobotany multiply rapidly, and the lack of specific knowledge of spores and pollen greatly handicaps the development of these phases of the science. The diagrams of Raistrick (64) and others definitely show that plant succession occurred in the ancient Carboniferous forests, and the prospects of exploring this field are by no means hopeless. The marked similarity of fossil spores and pollen between Europe and North America is an indication of widely spread similar or identical species. The use of microfossils to trace ancient forest migrations seems possible, and this information will be of real value to botanists and geologists alike. If the paleogeographic and paleoecologic phases become sufficiently clear there is reason to believe that they can be used in solving such problems as the Pennsylvanian cyclothems, the separation of late Mesozoic and early Tertiary formations, and the many problems of climatic sequence.

METHODS OF STUDY

The numerous laboratory techniques employed by investigators of peat fossils have been brought together and described in detail by

Erdtman (21). The methods of other paleobotanists have not been collected in this manner, and consequently must be sought throughout the literature. Field practices are generally not very well described in botanical literature, but those of the animal paleontologist are in a large part applicable to paleobotanical problems, and the book by Camp and Hanna (11) on "Methods in Paleontology" will be found useful. General procedure and methods are given below in brief form.

Collecting. In this phase of the work the type of sedimentary rock must be considered, for each requires different collecting methods. From consolidated rocks such as shale and coal the samples are taken at outcrops or from borings. The clastic sediments may be collected by direct digging or by boring with a soil auger or other type of sampler. Sand and clay samples may be recovered with well-drilling equipment, or if from lake sediments, by equipment designed by Ira T. Wilson (59). Peat and marls are collected by the use of the Davis (7) or Hiller (21) sampler.

The methods of sampling may be put into two general classes, channel and interval sampling. The former consists of cutting a block or column vertically through the deposit. The column then may be treated as a unit, divided into intervals of regular lengths, or divided into intervals determined by lithological benches. The use of lithological benches is frequently employed when coals contain pyrite or shale partings. Peat and silt can be collected easily at definite vertical intervals and are usually studied in that manner.

Preparation. The common methods of preparing plant microfossils are: (*a*) isolation, (*b*) thin sectioning, (*c*) nitrocellulose peels, (*d*) maceration, and (*e*) dispersal.

(*a*) Isolation. Frequently, large spores may be taken directly from certain coals, soft shales and glacial drifts. This is especially true of the large Devonian spores and the megaspores of the Pennsylvanian coals. Where considerable detrital material exists, separation may be accomplished by screening or by the use of a separatory funnel and bromoform (11). In the latter method the heavier materials sink to the bottom of the funnel and the spores float. Sometimes it is necessary to repeat the separation several times in order to isolate the fossils. Where large spores are being isolated from unweathered bituminous coal it is necessary to either pulverize or macerate the coal first and then proceed with screening operations.

(*b*) Thin sectioning. This method is well known. Briefly, the procedure consists of cutting a small block of coal or other rock, polishing one surface very smooth, cementing this face to a glass slide, and finally grinding the specimen to a desired thickness on a glass plate with carborundum grit. The method has been fully described by Thiessen (79). This manner of studying microfossils is frequently desirable when a comparison is being made of the various rock components and when morphological problems are being investigated.

(*c*) Nitrocellulose peels. This method has been applied largely to coal-ball studies, but has also been used on coals by Darrah (15), and by the writer on oil shales. The method consists of cutting the desired section of rock, polishing it smooth with carborundum powder, etching the surface with dilute acid, drying it carefully in warm air, applying one or more coats of nitrocellulose preparation to the surface, and when thoroughly dry, stripping off the peel and mounting it in balsam or other medium for microscope study. The greatest value of the peel method to microfossil studies is undoubtedly in coal-ball investigations. In these concretions there is a wealth of spore and pollen material that can be readily studied with peels. The method is especially suited to morphological studies, since one can frequently secure sections of spores or pollen by carefully grinding and etching the rock surface. Spore structures such as elaters are lost in maceration procedures but are retained by the peel method.

(*d*) Maceration. This is now the most common method of preparing consolidated sedimentary rocks for plant microfossil study. Various chemicals have been employed, depending upon the type of rocks studied. Sedimentary rocks containing calcium carbonate can be disintegrated by various strengths of hydrochloric acid, and those of siliceous composition are treated with hydrofluoric acid. Coals must receive special treatment depending upon their rank and state of weathering. Bituminous coals that are cut from a recently opened seam usually need treatment in Schultze's or other such macerating solution for a week or longer, but weathered coal macerates more easily. When the preliminary softening has taken place the coal is washed free of the solution, covered with a dilute solution of ammonium hydroxide and allowed to stand for several hours. Again the material is washed with water, and is then ready for study.

Coals react diversely in macerating solutions, and care must be taken to prevent being burned if the reaction is violent. The material should be frequently stirred and occasionally the progress of the maceration should be inspected by examining a sample of the coal under the microscope. Over-maceration of the spores and pollen readily occurs and will frequently destroy the diagnostic characters of spore or pollen walls. Maceration methods have been described (43, 85).

Some of the weathered coals and brown coals can be easily macerated with weak ammonium hydroxide, after which they must be washed with water until free of the odor of ammonium. Centrifuging the material greatly reduces the time of preparation, but care must be taken, for the fossils are easily broken.

(*e*) Dispersal. Unconsolidated sediments yield their spores and pollen quickly when dispersed by chemical means. Many methods have been published, but the most common now in use seems to be the KOH method described by Erdtman (20). It consists of boiling the peat sample in a 10% solution of potassium hydroxide, screening the humic mass, and concentrating the finer material by decantation or centrifuging. A second method has since been developed by Erdtman (21) which is much more drastic, but a greater concentration of microfossils is attained.

Staining. Most of the older microfossils do not require staining in order to be studied or photographed, for they are usually of various shades of brown or yellow. Fossils younger than the Paleozoic usually need to be stained in order to get the best photographic results. Staining of fossils in silt sediments also aids in readily distinguishing them from the inorganic particles. The stains most often used are safranine and gentian violet.

Mounting. There are several methods of mounting plant microfossils, but the two most frequently in use are the glycerin jelly and diaphane methods. The former is in some cases not as satisfactory as the latter, but its simplicity makes it possible to prepare a large number of samples in a very short time. Diaphane has been used with considerable success for permanent mounts of microfossils. It has the advantage of being somewhat more transparent than glycerin jelly. Other media that have proved useful are balsam, water-glass and Karo syrup. The last two should not be used if the material is to be kept over a period of years, for the media tend to become crystallized. Very large spores may be mounted dry on slides that are especially constructed for Foraminifera (11).

Photographs. The variety of plant microfossils is so numerous that if only line drawing illustrations are made, the task becomes very laborious. It is therefore desirable that some quick and inexpensive method of photographing be developed. Several types of cameras are available for this work, but if one must use other than 35-mm. film, this part of the work is very expensive. The film known as Panatomic X gives very satisfactory results, and when developed with a contrast developer such as D–11 or D–8, it is possible to enlarge the photographs to almost any size. Photographs of plant microfossils mounted on standard filing cards with information concerning the source of the material, the geological age, and a morphological description of the fossils, *etc.*, become of increasing value as the collection of microfossils grows. These photomicrographs become an absolute necessity when any extensive work is undertaken.

Classification. As in other branches of natural science there is a need for a clear understanding of taxonomic practice. Various systems of classification are now in use among plant micropaleontologists, but it is natural to expect that the system that attains the greatest proximity with phylogeny will be accepted. Some of the problems of classification will be discussed under the headings of various geological ages. However, it is necessary to state here that if order is to come out of the maze of taxonomic difficulties now existing in microfossil literature, there must be adherence to an international system of nomenclature.

MICROFOSSILS OF PALEOZOIC AGE

The rocks of the Lower Paleozoic era do not contain an abundance of plant spores, and those that are known must be considered with some degree of uncertainty. The earliest plants to produce spores were aquatic, and it is probable that the fossil spores reported from the Cambrian and Ordovician periods are all of that type.

Upper Cambrian spores with tetrad triradiate scars characteristic of the pteridophytes have been reported by Darrah (14) for the Ost-Gotland Swedish oil shale. If the spores prove to be Cambrian in age, they are the oldest showing that type of scar.

In the Ordovician shales of Illinois and Iowa, dark spore-like bodies are frequently observed. These have, to date, received little attention and their status is still questionable.

At present our understanding of Silurian spores is, like those of Ordovician age, very incomplete. However, since the discovery of Silurian fossil terrestrial plants in Australia, we can expect eventually to find a richer assortment of plant microfossils.

Devonian rocks contain numerous plant microfossils of which the best known type is a large dark-colored spore called *Sporangites*. It is a flattened disk-like body of about one millimeter in diameter. Frequently this fossil occurs in great quantities in the black Devonian shales of the Middle West and of Ontario, Canada. It is also known from Europe, South America and Australasia. The name *Sporangites,* as set up by its author, is difficult to retain, except as a term of rough description for dark, rounded, spore-like bodies. The name was first used by Sir Wm. Dawson (17) when he described some Devonian fossils as *Sporangites Huronensis*. At that time he considered them as the spores of *Lepidodendron,* but several years later, while describing two other species (18), he established the genus *Protosalvinia* and transferred *Sporangites Huronensis* to it. Dawson then supposed the spores to be related to the water fern, *Salvinia*. Today it is known that such a relationship is not the least probable. In 1875 Newton (50) described the Tasmanite and Australian white coal. Here he found spores similar to those of the American Devonian shales. The fossils were named *Tasmanites punctatus*. In the course of the descriptions of *Sporangites* and later of *Protosalvinia,* ambigous definitions were given. In an attempt to straighten out the situation, Schopf, Wilson and Bentall (71) have found it desirable to discard the name *Sporangites* as a *nomena ambigua,* and use Newton's *Tasmanites* for the large, thick-walled punctate spores of the Paleozoic rocks. The name *Sporangites* is retained as a convenient term for the larger undescribed spores.

Other types of spores occur abundantly in the Devonian rocks. Some of these have been found in organic connection with *Archaeopteris, Rhynia, Horneophyton* and others. Spores of the Devonian period have not received adequate attention, but should be a promising field for study.

From the Upper Paleozoic rocks hundreds of species of isolated and unassigned microfossils have been described, and eventually many more will be distinguished. In the coals of the Carboniferous and Permian periods the fossils consist mainly of pteridophyte spores and pollen of the more primitive gymosperms. One of the surprising facts in microfossil work is the marked uniformity of spore and pollen types that are encountered in widely separated regions. For example, many of the fossils of equivalent ages described from the coals of Russia, Africa and North America are almost identical.

Witham, 1833, appears to have been the first to have described spores in any coal (93). In his classical work on the "Internal Structure of Fossil Vegetables Found in the Carboniferous and Oolitic Deposits of Great Britain", he notes "traces of organisation". These "traces" were probably megaspores, but the identity of the structures were unknown to him. He was inclined to consider them the vessels of monocotyledons.

The first author to figure isolated fossil megaspores was probably Morris (47); however, in the description he regarded them as sporangia of *Lepidodendron* (*Lycopodites*) *longibracteatus*. Four years later Göppert (24) described and figured other megaspores under the name of *Carpolithes coniformis*.

In the subsequent few years other spore descriptions appeared in conjunction with cone studies. Hooker (32) described specimens of *Lepidostrobus* bearing microspores. Carruthers (12) described *Flemingites* sporangia which in reality are megaspores. Schimper (68) first described a fossil cone bearing *in situ* both mega- and microspores. The memoirs of Williamson (86) furnish numerous early descriptions and illustrations of spores both isolated and within their sporangia.

In 1884 Reinsch (66) published an important two volume work in which he figured over one hundred plates of micro-organisms from the coals of Russia and Saxony. Most of these are of spores or portions of spores. The work represents a careful and painstaking study, but unfortunately Reinsch thought the spores were algae, and the spore appendages, which are frequently complex, he considered to be parasites upon the algal cells. Reinsch's contribution to the microfossil content of coal was discounted because of his erroneous conclusions, but regardless of this incorrect interpretation, his work will remain a classic in plant micropaleontology. From Reinsch's investigations the only genus now recognized is *Triletes*.

Two years later Bennie and Kidston (3) read a paper before the Royal Physical Society, "On the Occurrence of Spores in the Carboniferous Formation of Scotland". In this paper they described and figured numerous megaspores without applying specific names to them. Bennie and Kidston at that time pointed out the mistaken ideas of Reinsch.

During the next two decades most of the fossil spore and pollen descriptions were given in conjunction with morphological studies of fructifications.

Some twenty years ago, Thiessen and his co-workers began a microscopic study of the eastern American coals with the object of correlating various seams by their organic content (79, 80, 81). The results were positive, but limited by the use of thin section methods. In thin sections of coal only a single view of a spore is obtained, which is usually not enough for the identification of most species. With the same objective, Slater, Evans and Eddy (77) began an examination of the English coals. Nineteen possible types were isolated and figured. All of their work, like Thiessen's was done with thin sections of coal, and consequently was limited in results.

In Germany and in England there appeared in 1931 and 1933 studies that have greatly stimulated the study of pre-Quaternary plant micropaleontology. R. Potonie (56) described a number of Paleozoic spores under the generic name *Sporites* of H. Potonie (53). Ibrahim (33, 56) and Loose (40, 56) in Germany began a comprehensive investigation of the taxonomy and geological range of fossil spores. Raistrick and Simpson (63) in England attempted the correlation of some Northumberland coals by spore content. In England the investigations emphasized the stratigraphic values of microfossils and no attempt was made to construct a binomial system of names that would be acceptable to international taxonomic practice. Much of the British work was confined to the study of the smaller spores, since they are considered as being more widely and uniformly spread by the wind and therefore more useful as horizon markers.

At the present time no general agreement exists concerning the classification of the Carboniferous spores and pollen. Raistrick and Simpson (63) devised a system of six spore groups, each of which is known by a letter of the alphabet, A to F inclusive. The various members of each group are then designated by a subnumber such as

A_1, A_2, A_3. The one good feature about the system is that it is a quick, easy method of treating large numbers of fossils both taxonomically and graphically. The continental workers, on the other hand, have established a binary system for the description of unassigned spores and pollen. The system is based upon the character of the exosporium and is definitely artificial. The genus *Sporites* of H. Potonie (53) was early used for spore descriptions, but this genus does little more than designate that the fossil is a spore. Ibrahim (33) proposed another system, which though more comprehensive is still very artificial. This new system proposed that all fossil spores be grouped according to their manner of dehiscence during germination. Three groups known as "Triletes", "Aletes" and "Monoletes" were established, and into these were placed spores whose dehiscence scar was triradiate (trilete), absent (alete) or single (monolete). Genera based upon spore wall characters were set up within each of the groups. Three spores differing in dehiscence scars but similar in wall ornamentation are treated as belonging to three separate genera; for instance, a "Triletes" spore with punctate walls belongs to the genus *Punctati-sporites,* an "Aletes" spore with punctate walls belongs to the *Punctata-sporites,* and a "Monoletes" spore with punctate walls belongs to the genus *Punctato-sporites.* Species within these genera are described in the usual manner.

In the above classifications no attempt was made to separate the large and small spores into morphological entities such as mega-, micro- or isospores. Several of Ibrahim's genera have genotypes that are megaspores, while most other members of the genera are microspores or isospores. In the construction of a natural classification of fossil spores it is important that all possible morphological characters be utilized. Therefore, since the large spores are usually megaspores and the small spores usually microspores or isospores, a more fundamental and natural classification may be made if spore size be included than if the classification be based on dehiscence scars alone. With an aim to summarizing the present knowledge of Paleozoic spores, and solving the taxonomic confusion that exists concerning these fossils, Schopf, Wilson and Bentall (71) have separated the large and small spores, and considered the validity of each pre-existing genus in accordance with the international rules of nomenclature. The results of this intensive search of the literasist mainly of spores, some of which resemble Paleozoic forms, but some are definitely like conifer pollen.

Another deposit that is of great interest occurs in the Petrified Forest of Arizona. Daugherty (16), while examining the microfossils, also discovered conifer-like pollen as well as several types of spores. One of the latter he refers to a new genus, *Equisetites,* because it closely resembles modern *Equisetum* spores.

Studies of Triassic rocks in the Salt Range of India have revealed spores that have been referred to the genus *Triletes,* and others tentatively to *Sporites* (51).

Jurassic and Cretaceous rocks contain some of the most interesting problems for the micropaleontologist, since during these periods the vegetation of the world underwent profound changes. The Jurassic period was a time of diminishing gymnosperm importance and the beginning of angiosperm floras, which by Cretaceous time dominated the Mesozoic landscapes. Simpson (76) has described an interesting collection of pollen from the Jurassic coal of Scotland. The seam has a rich pollen content made up principally of conifers belonging to the Abietineae. In addition, well preserved pollen of water-lily and possibly *Magnolia* are present. From other English sources spores of several Jurassic ferns, and megaspores belonging to *Triletes,* have been described (34, 48, 82). From the Lias of Greenland Harris (31) has secured many fern spores and conifer pollen associated with vegetative fossil remains. Likewise, the sedimentary rocks of Russia and India (49, 65) contain many microfossils, some of which may be considered pollen of conifers. The Jurassic rocks of North America have not been carefully explored for spores and pollen largely because there is a scarcity of nonmarine deposits.

The abundance of Cretaceous coals and suitable shale formations in North America make these rocks a fertile field for investigation. The Cretaceous coals and shales extend from the Gulf region northward through the interior of the continent to the Arctic. Other suitable Cretaceous deposits in North America are abundant but are not so continuous and widespread. The north-south distribution of Cretaceous deposits should be of great interest to the geographic studies of late Mesozoic floras. At present, only one American Cretaceous spore study has been published (46). In this paper Miner has described several spores which resemble those found associated with the Mesozoic ferns, *Gleichenites, Gleichenopteris, Laccopteris* and others. Cretaceous studies in Greenland (1, 45) have revealed numerous megaspore fossils that are now referred to the genus *Triletes*. Russian and South African deposits of Upper Cretaceous age contain considerable fossil pollen in addition to spores. Kirchheimer (37) has described a South African deposit containing pollen belonging to the modern families of Pinaceae, Betulaceae and Myricaceae.

MICROFOSSILS OF CENOZOIC AGE

The abundance of fossil spores and pollen in the Cenozoic rocks is remarkable. Early Tertiary deposits reveal the growing dominance of angiosperm pollen. Spores resemble those of the modern ferns *Thelypteris, Dryopteris* and *Lygodium*. Several lycopod and *Equisetum* types are also found, as well as a large quantity of fungal spores. The pollen is definitely modern in appearence, and both arborescent and herbaceous genera are represented. The gymnosperm representatives consist of pine, spruce, fir, cypress, hemlock, *Cycas, Zamia, Cedrus, Glyptostrobus, Cunninghamia, Sequoia, Ephedra* and others. The angiosperm trees include birch, oak, hickory, maple, basswood, elm, walnut, tulip, sour gum, willow and *Engelhardtia*. The herbaceous members present many difficult problems of identification but microfossils of the genera *Potamogeton, Typha, Smilax, Vitis, Myriophyllum, Rumex,* and of the families Araceae, Cyperaceae, Gramineae, Liliaceae, Caryophyllaceae, Ericaceae and Compositae have been recognized.

Some of the earliest investigations of Tertiary plant microfossils were made in Germany (6, 36, 54, 55, 57, 96). In those and subsequent studies several hundred species of fossil spores and pollen have been described and figured. The work indicates the richness of European deposits, and the great care taken to present the material makes the contributions valuable and outstanding. With the discovery of many problematical pollen fossils the question of classification has naturally appeared. This problem was solved temporarily by establishing the genus *Pollenites* (54) and treating the various fossil pollen as species within that genus. Such a lumping of forms is simply for description and does not promote the development of a natural system of classification. Potonie and other German workers have modified the procedure in cases where the phylogenetic affinity of the fossil is established. This is done by combining a generic name with an hyphenated suffix, as for example in *Tiliae-pollenites and Ulmi-pollenites.* Such a procedure is burdensome and unnecessary, for the most recognized Tertiary forms probably belong to existing genera or families. Others have preferred to place certain forms in modern genera (*e.g.*, 75, 95). Dr. Wodehouse (95) has made the suggestion that the term *Pollenites* be contracted to '*-pites*' and used as a suffix in the manner described as follows: "Thus a grain which is certainly that of *Pinus,* and which resembles most closely that of the living *Pinus Strobus,* is called *Pinus strobipites.* And the pollen of *Ephedra,* which is the first record of the genus in the Eocene in America, may be called *Ephedra eocenipites.* But, if the genus is not accurately or certainly known, the termination '*-pites*' is applied to the generic designation of the fossil grain instead of to its specific designation. Thus a grain which is known to belong to the Ericaceae, but the genus of which cannot be determined, is called *Ericipites longisulcatus,* for example, the specific name having reference to the length of its furrows. And a grain that matches the living species of *Smilax* but also matches equally well those of some other genera is called *Smilacipites molloides,* for example, its specific designation referring to its further resemblance to the grains of the living *Smilax mollis.* If at some later date any of those genera which bear the termination '*-pites*' should become more closely defined or proved to be accurately determined, the termination may then be transferred to the specific names. The advantages of this system are that complete freedom is allowed in the use of descriptive adjectives as specific names without the introduction of trinomials, some idea is conveyed of the closeness or reliability of the determination, and always is shown the fact that the determination is based on a fossil pollen grain". The suggestions have real merit, but the transfer of the suffix '*-pites*' may easily inject taxonomic errors into the literature.

Oil shales long have been known to contain much organic material and those in Colorado known as the Green River shales (Eocene) are unusual in the amount of spore and pollen fossils they contain. Bradley (8) has described the geology of the deposit and Wodehouse (94, 95) the pollen. Forty-three species in thirty-four genera have been described, only one of which cannot be assigned to a living genus or family. Twenty-one genera are recorded for the first time in the Green River flora, which is testimony of the manner in which microfossil studies may supplement the macrofossil record. Wodehouse also states that there still remain possibly twice as many undescribed forms in the material that he studied. Wilson and Webster (unpublished) have studied Wyoming coals, also of Green River age, and have distinguished nearly two hundred species of spores and pollen. Many of the forms are identical with those described by Wodehouse. In each case the fossils indicate that the Eocene forests of the Middle Rocky Mountain region were to a large degree similar in generic composition to those now existing in the Northern and Middle Atlantic states. Several interesting additional forms, such as *Ephedra, Glyptostrobus, Cunninghamia* and *Engelhardtia,* have been found in these microfossil floras.

From the Tertiary lignites (Eocene) of west Scotland, Simpson (75) has described pollen fossils belonging to twenty genera. These show a marked resemblance to the East Asiatic flora and certain forms now confined to Africa and America.

The younger Tertiary deposits of North America have not been investigated for plant microfossils, but numerous published studies in Germany (57, 96) indicate that the flora of Europe had become very modern in aspect.

With the coming of the continental ice sheets and the uplift of certain regions of the earth, the Tertiary period came to a close and the Quarternary period began. The microfossil literature for the Quaternary period has been reviewed in this journal (9, 72). Since the publication of Cain's review, the work by investigators in North America may be briefly summarized as (*a*) extending the geographic range of peat studies, (*b*) investigating the interglacial peat deposits, (*c*) correlating of pollen spectra by stratigraphic methods, (*d*) tracing the postglacial migration of forest elements, and (*e*) testing out theories of climatic sequence.

The geographic range of peat studies has been greatly extended and intensified within the United States and Canada. Those workers most active in the field at the present time are Cain, Deevey, Hansen, Knox, Potzger, Sears, Webster, and Wilson. The literature is too extensive to list here, but may be found in the "Pollen Analysis Circular" edited by Dr. Paul B. Sears of Oberlin College.

Buried interglacial silts and peats have revealed much concerning the distribution of our forests during the glacial period. This is fortunate, for the peats that were present as surface deposits on the older glacial drift plains have largely disappeared as the result of oxidation and erosion. Lane, Voss, and others (39, 84) have described the fossils of the Pleistocene peat deposits of the middle west, Hansen (25) has worked on the west coast, and Knox (67) in the east. The most complete series of interglacial peats that have been described are from the middle west, which is to be expected, since there the drifts are better represented. All of the interglacial deposits except the earliest, the Aftonian, have to date indicated that the Pleistocene forests of northern United States consisted mainly of spruce, fir and pine (39, 90). At the start and end of the Aftonian stage the forests of the middle west also consisted of spruce, fir and pine, but during the middle of the period the forests of Iowa, southern Minnesota and probably Wisconsin contained considerable oak, hickory, maple, basswood and elm.

Correlation studies, though still not abundant, indicate that much can and will be done in the near future. When peat workers attain greater knowledge of Pleistocene geology we can expect more studies of this type. Voss (83) has made a comparative study of bogs on Cary (3rd Wisconsin) and Tazewell (2nd Wisconsin) glacial drift in Illinois. He found that the forest succession on these two closely associated glacial drifts was much alike. Numerous recent papers by Dr. Potzger and his students (58, 60, 61) indicate by the constancy of the pollen curves the stratigraphic use that can be made of pollen spectra within a region. Wilson (87) applied stratigraphic methods to peats associated with various glacial lake stages in northwestern Wisconsin. By these methods the forests were shown to have developed according to the usual order of succession (spruce, pine, deciduous) upon each succeeding plain as the lake levels became progressively lower.

The geographic density of peat studies in North America is now sufficient to allow summaries of various types to be drawn. One of these is the postglacial migration of the forest elements. From 111 peat deposits in the eastern and middlewestern states and northeastern Canada, Sears (74) has traced the postglacial migration of *Quercus, Tsuga, Carya, Fagus and Tilia*. In the northeastern part of the area, the above genera appeared in peat profiles in the following order: *Quercus, Tsuga, Fagus, Carya* and *Tilia*. In the southwestern part, *Quercus, Carya, Tilia, Tsuga* and *Fagus* is the order of appearance. Important centers of dispersal appear to have existed in the southeastern part of the area and in Wisconsin, possibly in the Driftless Area. Sears correlates the migration of the trees with climatic fluctuations, and points out that *Carya* pollen occurs as fossils in peat, north of the present range of that genus. This latter distribution has received additional discussion (91, 92, 10). The former authors contend that though fossil *Carya* pollen is small in its relative abundance when it occurs considerably north of its present range, it is present only in the higher middle levels of the peat deposits and is consistently absent from the surface layers; therefore, the genus should be considered as having had a more northern range. Cain contends that the fossils probably represent pollen that was wind-borne from a more southern location.

Forest sequence studies (73) from pollen spectra in Illinois, Indiana, Michigan and Ohio suggest that there were two general periods of retrogression in that region. They are assumed to have been due to climatic causes which produced a less favorable water balance. For northern Wisconsin, Minnesota and western Ontario there is good evidence for only one forest retrogression (58, 60, 91). This difference in pollen evidence may prove later to be related to glacial history, since the bogs described by Dr. Sears are located on Cary drift (3rd Wisconsin), while the more northern deposits are developed on the Mankato drifts (4th and 5th Wisconsin).

Climatic inference has long been an important part of Quaternary micropaleontology. At present there are several theories of postglacial climatic history. Smith (78) has attempted the correlation of 148 eastern North American pollen profiles with a view to their climatic inference. He has come to the conclusion that the major European climatic periods (preboreal, boreal, Atlantic, subboreal, subatlantic) are evident in the profiles from the entire eastern North American region. He further states: "The above major climatic periods are indicated in the profiles from the entire region. The profiles from the region are also consistent in showing minor variations of climate within these major periods. These minor variations, occurring almost rhythmically, show that while the European divisions of postglacial climate outlined above apply in a general way to the eastern North American profiles, postglacial climate in eastern North America is perhaps better characterized as a series of climatic pulsations of more or less humid periods".

Sears (74) has suggested the following scheme of climatic changes for eastern North America:

V. The present—probably cooler and with more available moisture than in IV.
IV. A warm dry period—maximum of oaks and hickories, minimum of beech.
III. A more humid, also dry, period—maximum of beech, and, in places, of hemlock.
II. A dry, probably warmer, period—maximum of pine, often with oak.
I. A moist cool period—maximum of fir and spruce.

In the Lake Superior region of northern Wisconsin, Minnesota and Ontario, Wilson and Webster find a sequence of forest changes that they tentatively consider as supporting von Post's view (52). This hypothesis postulates a tri-partite division of postglacial climate: first, a period of increasing warmth; second, a period of maximum warmth; and third, a period of decreasing warmth. The pollen spectra of the Lake Superior region as interpreted by Wilson and Webster agree with periods V and IV of Sears. Period III may later prove to be comparable to the lowest levels of the pollen spectra in the Lake Superior region. Work by Wilson (unpublished) in south central Ohio agrees with Sears' five-fold division of postglacial climate. In southern Ohio the peat pollen record began with the recession of the Tazewell (2nd Wisconsin) ice, while in the Lake Superior region the record began only after the melting of the Later Mankato (5th Wisconsin) ice. Just how much time elapsed between these substages of the Wisconsin glaciers is not known, but the difference in pollen spectra on the drifts would suggest that the time interval was not short. Potzger (58) does not favor the assumption that the present is a period of decreasing warmth. He states: "so many concrete evidences have been published in recent years which show definitely the opposite of such a conception that one reads such postulations with suprise". It might be stated that for each citation given by Dr. Potzger as against the idea, there are others, some of which are by the same men but more recent, that favor the theory of decreasing climatic warmth (13, 42).

For the Spokane, Washington and northern Idaho regions, Hansen (26) has postulated a fivefold postglacial sequence of climate. He states that the initial period was cool and medium dry; second, there was increasing warmth and dryness; third, further drying and warming; fourth, increasing coolness and moisture; and fifth, coolness and medium dryness. For western Oregon and northwestern California, he indicates (28, 29) that there appears to be some agreement with the von Post scheme of climatic succession. For the insular region of Washington he finds (30) that there is little evidence for climatic trends, and that it is probable that the ocean has served to maintain an equable climate during postglacial time.

SUMMARY

1. Spores and pollen have been abundantly preserved as microfossils in certain sedimentary rocks from Paleozoic time to the present.

2. Objectives in pursuing the study of plant microfossils are: (*a*) to establish the biological affinities of isolated fossil spores and pollen, and to determine the phylogenetic relationships; (*b*) to use spores and pollen as indices in the stratigraphic study of rocks; and (*c*) to aid in the understanding of paleogeographic and paleoecologic problems.

3. The methods of microfossil study are briefly discussed under the various phases of collecting, preparation by isolation, thin sectioning, nitrocellulose peels, maceration, and dispersal, staining, mounting, photographs and classification.

4. Plant microfossils of the Paleozoic rocks consist mainly of pteridophyte spores and the pollen of primitive gymnosperms. Several hundred species have been described. Attempts to use microfossils as horizon markers have been successful.

5. The early Mesozoic plant microfossils are predominantly pteridophyte spores and gymnosperm pollen, but those of the upper Mesozoic rocks contain, in addition, quantities of angiosperm pollen.

6. Cenozoic microfossils consist largely of angiosperm pollen, many of which belong to modern genera.

7. The recent Quaternary microfossil studies in North America indicate that pollen spectra may be used for stratigraphic correlation of interglacial and postglacial sediments. The migration routes of certain forest elements have been traced. The theories of climatic change in postglacial time based on microfossil studies show some refinement, but are as yet tentative.

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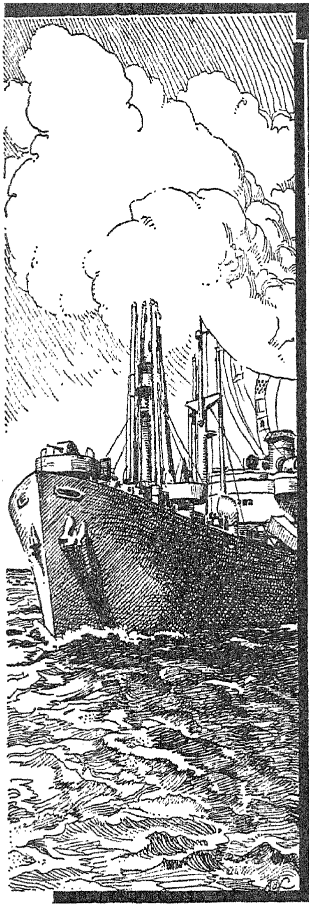

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SOIL MOISTURE IN RELATION TO PLANT GROWTH

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SOIL AND ITS PHYSICAL CHARACTERISTICS

Some knowledge of the principles governing the distribution and availability of soil moisture is essential to a proper understanding of plant-water relations. Soil, considered in relation to plant growth, is not a mere mass of "dirt", but a complex system consisting of varying proportions of four principal components: mineral materials, organic matter, water and solutes comprising the soil solution, and air. While the amounts of mineral materials and organic matter vary but little in a given soil, the amount of water fluctuates over a considerable range and the amount of air varies approximately inversely with the water content. Furthermore, the proportions of oxygen and carbon dioxide in the soil atmosphere vary with depth, season and other factors (10). In addition to these four components, soil usually contains numerous living organisms, such as bacteria, fungi, algae, protozoa, insects, earthworms and other small animals. These may directly or indirectly affect plant development, and the burrows of earthworms and other small animals provide passageways which facilitate the downward percolation of water through impermeable soil strata.

The characteristics of a soil depend chiefly on the texture or size of mineral particles, the structure or manner in which these particles are arranged, and the amount of organic matter incorporated with the mineral matter. On the basis of texture, soils are usually classified as gravel, sand, loam or clay. The last three classes are most important with reference to plant growth and will be discussed briefly. More detailed discussions can be found in various soils texts (7, 52, 62, 81).

The least complex soil is a sand, which by definition contains less than 20% of silt and clay and is composed largely of simple rock particles of comparatively large size. Such a soil forms a relatively simple capillary system with large pores or air spaces which insure good aeration and free movement of gravitational water. Sandy soils are relatively inert, both chemically and physically, quite loose and non-cohesive, and have a very low water-holding capacity.

Clay soils are at the other extreme with reference to size of particles, consisting of 30% or more of clay particles, most of them of colloidal or near colloidal dimensions. These particles are usually aggregated together in complex granules which swell and become sticky when wetted. Because of the large proportion of particles of colloidal size in clay, water and minerals are held in much larger quantities and in a more complex manner than in sand. Most soils owe many of their chemical and physical properties to the clay fraction which they contain. The clay particles, because they are flat and plate-like, possess not only the maximum external surface, but also very high cohesive forces. They are usually negatively charged and carry a shell of cations and associated water molecules. The surface possessed by even a small volume of such particles is tremendous. A cubical sand grain one millimeter on the edge has a surface of only six square millimeters, but if divided into cubes of colloidal size, 0.1 micron on the edge, the total surface resulting would be 60,000 square millimeters. Because of their plate-like shape clay particles have even greater surfaces than cubes or spheres of similar volume. Their extensive surfaces enable clay soils to hold much more water than sandy soils, but since the pores are much smaller gravitational water drains off more slowly and they are sometimes poorly aerated.

Loam soils contain more or less equal proportions of clay and sand and therefore have properties which are intermediate between those of clay and sand. They are most favorable for plant growth because they hold more available water than sand, and are better aerated and easier to work than clay.

Soil structure, or the arrangement of soil particles, is important because of its relation to pore size. Soil porosity refers to the portion of the soil volume not occupied by solid particles, but by air and water. It usually amounts to about half of the volume, generally comprising somewhat less than 50% of the total volume in sand and somewhat more than 50% in clay, though exceptions to this exist (94). The total pore space is not so important as the size of the pores. That portion of the pore space composed of large pores from which water usually drains by gravity and which is therefore normally filled with air is termed the non-capillary porosity to distinguish it from that part of the pore space which is normally filled with capillary water. The large pores and large non-capillary porosity of sandy soils result in better drainage and aeration, but also result in a lower water-holding capacity than in clay soils which have a large proportion of smaller capillary pores. An ideal soil is said to be one which has the pore space about equally divided between large and small or non-capillary and capillary pores (7). Such a soil has enough large pores to permit adequate drainage and aeration and enough small pores to give adequate water-holding capacity. In clay, treatments tending to promote granulation produce larger pores, resulting in a more favorable medium for root growth. The dense mat of roots produced by grass sod seems to promote granulation and good soil structure, while cultivation generally produces the opposite effect (7, 11). The direct effects of root penetration through the soil, followed by their death and decay, is to open up numerous channels and thus to materially increase soil porosity and penetration by air and water. Earthworm activity probably has similar effects.

Soil structure is also affected by the kinds of cations present. According to a summary prepared by Veihmeyer (95), there is evidence from laboratory experiments that various properties, including moisture equivalent, permeability, hardness of crumbs and cohesiveness, are increased by substitution of sodium or potassium ions in the exchange complex and decreased by substitution of calcium or hydrogen ions. Most investigators seem to think these results also apply to field conditions, though few data are available from field tests. Eaton and Horton (30) reported that the wilting coefficient and moisture equivalent of soils partially saturated with sodium were higher than those of the same soils treated with calcium, if most of the soluble electrolytes was first removed. They state that it has been frequently observed that permeability of soil is reduced by irrigation of land with water containing a higher proportion of sodium than of other bases. This is important in semi-arid regions where rainfall is too light to remove the salts concentrated in the surface layer by evaporation of irrigation water.

Appreciation of the importance of soil structure has raised questions concerning the propriety of determining such soil properties as water-holding capacity, moisture equivalent and permanent wilting percentage on disturbed soil, as is usually done. Soil samples are usually dried, pulverized and passed through a sieve, thoroughly destroying the original structure, before they are subjected to various tests. In recent years a number of methods have been described for obtaining soil samples without disturbing their structure. One type of sampler (21) provides undisturbed soil masses for volume weight and water-holding capacity determinations and for permanent wilting percentage determinations.

The organic matter or humus in soil represents that portion of plant remains, chiefly lignified material, which is most resistant to decay. It resembles clay in some respects, being colloidal with negatively charged micelles surrounded by shells of cations, and shows even more chemical and physical activity than does clay. Because of its very great surface it holds much water, and addition of organic matter to sandy soils increases their water-holding capacity. Its addition to clay may improve the latter's structure and workability, but usually has much less effect on available water content than is commonly supposed because the water-holding capacity of clay is already comparatively high and the unavailable water content of organic matter is very high. A mixture of one-half clay and one-half peat moss by volume held only about 25% more available water than pure clay, while a mixture of one-half quartz sand and one-half peat moss held about 800% more water than pure sand (34). Apparently it is difficult to make any important changes in the available water content of soils under field conditions. In California (96) it was found that addition of as much as 200 tons per acre of manure did not appreciably increase the content of water available to plants in sand, loam or clay soils. In New York (40) additions of 8 and 16 tons per acre of manure for 27 years did not significantly increase the available water-holding capacity of Chenango loam, but did significantly increase the available water-holding capacity of Chenango fine sandy loam.

SOIL MOISTURE CLASSIFICATION

The classification of soil moisture most familiar to plant scientists is the simple system of Briggs (12) with the addition of water vapor as suggested by Lebedeff (57). This system divides soil moisture into four classes:

a) gravitational water, which occupies the larger pores of the soil and drains away under the influence of gravity; it is often injurious to plants if drainage is too slow;

b) capillary water, which is held by surface forces as films around the particles, in angles between them and in capillary pores; capillary water moves slowly in the form of liquid from thicker to thinner films, and is the only important source of water for most plants;

c) hygroscopic water, which is held on the particles by surface forces and moves in the form of vapor; the moisture remaining in air-dry soil is usually regarded as hygroscopic water and is generally unavailable to plants;

d) water vapor, which occurs in the soil atmosphere and moves along vapor pressure gradients; it is probably not used directly by plants.

Such a classification must be regarded as somewhat arbitrary in the light of present-day theories of soil moisture because there really is no sharp boundary between these different classes of soil moisture (97). Under certain conditions capillary water may become gravitational water and hygroscopic water may merge into capillary water. This classification seems sufficiently useful, however, especially to plant scientists, to retain it in spite of its arbitrary nature. A brief discussion of the various types of water follows.

Gravitational water. For a short time following a heavy rain or irrigation the soil may be completely saturated with water, the air in it having been displaced from the non-capillary pore spaces between the particles. Under the influence of gravity most of this free water soon percolates downward through the soil toward the water table, unless prevented by some barrier such as a hardpan layer. Within two or three days after a rain all the gravitational water usually drains out of at least the upper horizons of the soil, and the pore spaces become refilled with air. If the soil remains saturated with gravitational water for several days serious injury to root systems may result from lack of oxygen and accumulation of excess carbon dioxide. Obviously gravitational water is therefore of little direct value to most plants and even may be detrimental.

Capillary water. After the gravitational water has drained away a soil is said to be at its "field capacity". The water remaining exists as films around the particles, in the angles between the particles, and in the smaller capillary pores. Much of this water is held rather loosely and is readily available to plants. Some of it, often termed "inner capillary water", is held by the colloidal material and in the smallest pores and is relatively unavailable to plants. As previously stated, the finer the texture of the soil the more surface is exposed and the more capillary water it will hold. Since capillary water moves slowly it is relatively unavailable unless the roots actually come into contact with it. Puri (74) claims that the lower limit of water available to plants is determined principally by the number of pores too small to be penetrated by root hairs. In general, movement of capillary water from moist to dry soil is quite slow, and the significance of this with respect to absorption will be discussed later.

Hygroscopic water. The water remaining in a soil in equilibrium with a partly saturated atmosphere is usually termed "hygroscopic water". It is held in a very thin film perhaps 15 to 20 molecules in thickness. The upper limit of hygroscopic moisture is generally supposed to be the moisture content of soil in equilibrium with saturated air, but soil kept in contact with moist air over a long period may accumulate so much water that it actually becomes saturated (52). This illustrates the difficulty of sharply distinguishing between these two classes of soil moisture. Hygroscopic water, if the term is used to refer to the water in approximately air-dry soil, is obviously unavailable to plants.

SOIL MOISTURE TERMINOLOGY

The literature dealing with soil moisture contains numerous special terms. Many are of interest only to soils men or engineers, but a number of them are frequently used in discussions of plant and soil moisture relations. Among the terms frequently encountered in discussions of plant and soil water relations are "capillary potential, pF value, capillary capacity, maximum water-holding capacity, field capacity, hygroscopic coefficient, wilting coefficient, wilting point, permanent wilting percentage, wilting range, moisture equivalent, readily available water, relative wetness, water-supplying power".

The "capillary potential" or "pressure potential" of a soil, often designated by the Greek letter psi (ψ), is a measure of the attractive forces with which water is held by the soil, or an expression of the work done in moving water against the capillary forces of the soil. Buckingham (17), who originally proposed the term, expressed it as the height in centimeters of a water column which would exert sufficient pressure to move water in a soil at a given moisture content. It may also be expressed in gram centimeters per gram or ergs per gram. The potential of free water, which is the base or reference value from which the potential of soil moisture is measured, is regarded as zero. Since the potential of water in unsaturated soil is lower than that of free water, the potential of soil water must be regarded as having a negative value. The capillary potential increases negatively with decreasing moisture because more energy is required to move water in dry soil than in moist soil. Since it is negative the potential is sometimes expressed as a tension in terms of the height of the water column required to produce it, as for example 250 or 500 centimeters of water, or the corresponding height of a mercury column.

Recently use of the thermodynamic function of "free energy" has been proposed to deal with soil moisture problems (31, 32, 83). It is a more generalized quantity which includes the capillary potential as a special case. The latter deals only with the energy relations resulting from the tension or pressure existing in soil moisture, and excludes the effects of dissolved salts and the possible adsorption of water molecules around soil particles. These are included by the quantity "free energy". The capillary potential is approximately equal to the free energy value when the concentration of solutes is negligible. The free energy and the capillary potential can be measured or calculated from measurements made in various ways, such as by determining the vapor pressure or the freezing point, and by use of the centrifuge, dilatometer, osmotic membranes, tensiometers and pressure devices. Tensiometers (77) are essentially porous clay cups, similar to autoirrigators, connected to a mercury manometer or vacuum gauge which indicates the negative pressure or tension when the water in the cup is in equilibrium with the soil moisture. The capillary potential at various moisture contents is sometimes measured in the laboratory by placing the soil in contact with a porous plate attached to a vacuum line. The soil is subjected to any desired suction and the moisture content is then determined. Unfortunately these methods operate only to a potential equal to one atmosphere or less, so the higher potentials existing in drier soils are usually calculated from their vapor pressures. Richards (76) has recently developed a method of applying pressure which gives potentials under pressures up to 16 atmospheres or more.

The energy concept of soil moisture is becoming more popular because it indicates the condition of the soil moisture independently of texture, structure or composition. The moisture relations of very diverse soils can therefore logically be compared because, for example, the capillary potential, pF, or free energy of the soil water at the permanent wilting point is approximately the same in all soils.

"Soil pF". Schofield (85) proposed the use of the logarithm of the capillary potential expressed in centimeters of water. He termed this the pF scale by analogy with the pH scale, "p" referring to its logarithmic character and "F" indicating that it refers to the logarithm of the free energy difference. The chief advantage of this scale is that it permits the entire range of soil moisture tension to be shown on one compact scale, although the equivalent water column is almost 10 kilometers (1,000,000 cm.) at equilibrium with 50% relative humidity (pF 6) and about ten times greater at oven-dryness (pF 7). The moisture equivalent is at a pF of about 2.7, the wilting percentage at about 4.2. Water may be regarded as moving along a pF gradient from regions of low pF to regions of higher pF. The pF can be directly determined down to about pF 3 by applying suction to a thin layer of soil, and values for drier soils have been calculated from vapor pressure and freezing point depression data or more recently by application of direct pressures up to 16 atmospheres (76).

The "hygroscopic coefficient" is the moisture content of the soil in equilibrium with an atmosphere of known relative humidity—usually a nearly saturated atmosphere. According to Keen (52) and others, the experimental difficulties inherent in making such determinations render them of very doubtful value, although at relative humidities below saturation reproducible results can be obtained. This value generally is of minor interest to plant scientists.

The "maximum water-holding capacity" is the water held by a thin layer of saturated soil. The soil is placed in a shallow metal container with perforated bottom and allowed to stand in water until saturated. This gives measures of pore space, specific gravity and expansion on wetting, and Keen (52) recommends it as a measurement useful to the soil scientist. The method of draining surplus water will affect the results obtained, and the results may also vary depending on whether the measurement is made on pulverized soil or a soil mass with undisturbed structure.

The "maximum capillary capacity" of a soil is the maximum moisture content held against gravity and is therefore essentially similar to the field capacity.

✓The "field capacity" of a soil is the moisture content after the gravitational water has drained away (101), and it is therefore essentially equal to the capillary capacity. Most soils are at their field capacity within two or three days after a rain or irrigation (103). Soil samples in short columns allowed to drain over sand probably reach approximately their field capacity within a few hours. This is not a true equilibrium value, but only a condition of such slow water movement that the moisture content does not change appreciably between applications of water. While most soils reach their field capacity very quickly, the presence of a water table near the surface will greatly prolong the time required for drainage, and if the soil is saturated to a depth of many feet, drainage of the surface layer to field capacity will be much slower than if only the top few feet are wetted. A fine-textured soil overlying a coarse-textured soil will also have a higher field capacity than a uniformly fine-textured soil (103).

The term "moisture equivalent" was introduced by Briggs and McLane (13) to denote the water content of soil which has been subjected to a centrifugal force of one thousand times gravity in a soil centrifuge. The precautions necessary to insure accurate results have been discussed (*e.g.,* 105). The moisture equivalent has been found to be closely related to the field capacity of fine-textured soils but not of sands (101). Work and Lewis (108) found that the moisture equivalent of certain Oregon soils was not equal to the field capacity, and such a relation should not be assumed without actual determinations. The ratio of field capacity to moisture equivalent of certain West Virginia soils is unity in the vicinity of a moisture equivalent of 21% more than unity for soils with moisture equivalents below 21% and less than unity for those with moisture equivalents above 21% (16). Although the equipment is expensive, determinations are so easily made that the moisture equivalent is one of the most frequently determined soil constants.

"Permanent wilting percentage". The moisture content of the soil at the time when the leaves of plants growing in that soil first become permanently wilted has been variously designated as the "wilting point", "wilting coefficient", "wilting percentage", and "permanent wilting percentage". It will be designated as the "permanent wilting percentage" in this paper. Briggs and Shantz (14) first emphasized the importance of this soil moisture content with respect to plant growth and termed it the "wilting coefficient". Their procedure was to grow seedlings in glass tumblers of soil sealed with a mixture of paraffin and vaseline. When the leaves wilted and did not recover over night in a moist chamber the moisture content of the soil was determined by oven-drying a sample at 105° C. and calculating the moisture content as a percentage of the dry weight. Briggs and Shantz stated that this marked the lower limit of soil moisture available for growth, but not the lower limit of soil moisture available for the plants. Although absorption is too slow for growth at moisture contents below the wilting point, plants are able to absorb water from the soil until it is approximately air-dry or until they have died of desiccation. Permanent wilting, according to Briggs and Shantz, does not mark any definite limit in the movement of water from soil to plant, but simply marks the moisture content at which absorption becomes too slow to replace the water lost by transpiration, resulting in wilting. Shull (91) came to similar conclusions regarding the cause of wilting, and these views have been substantiated by more recent investigations and are generally accepted at the present time.

The moisture content of the soil at the time of permanent wilting might conceivably be affected by the species and condition of the plants used in its determination and by the environmental conditions under which it is determined, as well as by the soil texture. Briggs and Shantz (14) concluded, however, that soil texture is the only factor materially affecting the moisture content at permanent wilting. Age of their plants did not materially affect the values, as the same results were obtained with seedlings and well grown grass plants. Plants grown with different amounts of soil moisture wilted at the same moisture content, indicating that drought resistance had not been increased by growing the plants in dry soil. Contrary to previously accepted views, they found no important differences between different species of plants in regard to their ability to reduce the moisture content of the soil before wilting. The differences observed between various species of crop plants were too small to explain differences in drought resistance, and even these small differences were believed to result from differences in root distribution rather than from differences in forces bringing about water absorption. Similar results have been obtained with a number of crop plants (20). The results and conclusions of Briggs and Shantz have been criticized for various reasons (15, 19, 24, 72, 90), but they are substantiated by more recent investigations. Briggs and Shantz found that while all species wilted at approximately the same moisture content in a given soil there were considerable differences between species in ability to survive in soil below the wilting point. Some species died soon after wilting, while others survived for long periods in a partially wilted condition. Some of the criticisms of Briggs and Shantz' work probably result from failure to differentiate between the early stage of wilting used by them as an end point and later stages approaching the ultimate wilting point of Taylor, Blaney and McLaughlin (92) and Furr and Reeve (37).

The type of plant used in determining the wilting percentage does have some bearing on the reliability of the results. The first requirement is a well-developed fibrous root system which so completely permeates the soil that the moisture content is evenly reduced throughout the soil mass. The leaves must be of a type which show wilting clearly, and plants with heavily cutinized leaves, such as pine needles, are therefore unsatisfactory. The writer found that the wilting point of a sandy loam determined simultaneously with sunflower, black locust and pine seedlings appeared to be highest with sunflower, and lowest with pine seedlings. This probably does not represent any difference between these species in ability to reduce the moisture content, but resulted from the greater difficulty in determining when the pine seedlings had begun to wilt. Moinat (68) suggested that determination of the wilting point may be in error because of the leaves removing water from the stem or other parts of the plant after the soil is really at the wilting point. This would be a negligible factor when oats or other grasses are used as indicators. It probably is also a minor factor if the wilting process is terminated when the first pair or two of leaves have wilted.

A lively controversy arose concerning the influence of atmospheric factors on determination of the wilting percentage. Briggs and Shantz (14) made most of their determinations in a greenhouse where transpiration was very moderate but considered that the values were not materially affected by atmospheric conditions such as humidity or by moderate changes in light intensity. Two other investigators (15, 19) found that plants growing in the open wilted at a higher soil moisture content than plants growing in shade with a lower rate of transpiration. One of these (19) concluded that "for a series of plants grown in any one soil and wilted under a number of aerial conditions, as many different soil-moisture contents are obtained as there are sets of conditions". In another experiment (90) in which several species were wilted under various degrees of shading, it was found that the moisture content of the soil at permanent wilting was lowest in the shade and highest in full sun. Veihmeyer and Hendrickson (102) found the wilting percentage of a particular soil to be remarkably constant, regardless of size of container, species of plant, season of the year, or degree of exposure. Sunflower plants were wilted in the spring and during the hot dry weather of late summer. Some seedlings were allowed to wilt in a moist chamber in a greenhouse, others in a whitewashed greenhouse and still others in the open. The average wilting percentage was the same in all instances, although the rate of evaporation was several times as high when some plants wilted as when others wilted. Furr and Reeve (37) obtained similar results. Discrepancies between the conclusions of various investigators may have been caused in part by differences in opinion as to what constitutes the onset of permanent wilting.

Sachs (84) seems to have been the first to study the effect of soil texture on the availability of water to plants. He found that when tobacco wilted in sand, loam and a mixture of humus and sand, the moisture contents at the time of wilting were 1.5%, 8.0% and 12.3%, respectively. Apparently little attention was paid to this situation until publication of the very extensive work of Briggs and Shantz (14). They found the moisture content at permanent wilting to vary from 1% in sand to 25% in clay and even higher in soils containing much organic matter.

In addition to actually determining the wilting point, Briggs and Shantz attempted to calculate it from the moisture equivalent. They found that for their soils,

$$\text{Wilting coefficient} = \frac{\text{moisture equivalent}}{1.84 \pm 0.013}.$$

This value has frequently been used, but subsequent work by several investigators show that such a relation does not always exist. In an investigation of 60 California soils (101) the ratio was found to range from 1.4 to 3.8. The ratio of moisture equivalent to wilting percentage in three soils on the Duke Forest ranged from 1.57 to 5.65, varying with soil type and horizon (29). Briggs and Shantz also attempted to relate the wilting coefficient to the hygroscopic coefficient, the moisture-holding capacity and the mechanical analysis. The usefulness of any such cross-relating of values is obviously very doubtful, since the relations are not the same in all soils. The moisture content at permanent wilting appears, however, to be logically and consistently related to certain other values. The permanent wilting percentage of most or possibly all soils falls at about pF 4.2 and at the moisture content existing after application of a pressure of 15 atmospheres. The moisture content of a soil at permanent wilting can be determined most reliably by direct observation. Since the term "wilting coefficient" has often been applied to indirect determinations, it would seem preferable to use the term "permanent wilting percentage" to indicate determinations made by the direct method (100).

Wilting, of course, does not begin at a specific moisture content, but it does begin within a very narrow range of soil moisture for which the value given as the permanent wilting percentage is the average. The more care taken in making the determinations, especially in bringing all plants [illegible] the same stage of wilting, the more consistent the results. Since the permanent wilting percentage is so stable for a given soil and since it accurately indicates the lower limit of soil moisture available for plant growth, it is perhaps the most important of all soil constants for the plant scientist. Detailed descriptions of the methods used in precise research have been given by several experimenters (14, 37, 108). The writer has obtained satisfactory results with less elaborate methods, merely growing oat or sunflower seedlings in heavily paraffined pint ice cream containers until they are three or four inches high, sealing the soil surface with paraffin and allowing the containers to stand on a partly shaded greenhouse bench until the lower leaves remain visibly wilted over night.

"Wilting range of a soil" is a term applied to the range of soil moisture from the first permanent wilting of the basal leaves of sunflowers to complete permanent wilting of the entire plant (92). Furr and Taylor (38) and Furr and Reeve (37) have presented data on this range, and Furr and Reeve give detailed instructions for its determination. They use the terms "first permanent wilting point" and "ultimate wilting point" to designate the upper and lower limits of the wilting range and like Briggs and Shantz regard the first permanent wilting point as the lower limit of soil moisture available for growth. The moisture in the wilting range, while unavailable for growth, is available for survival, and the proportion of the total available soil moisture which is within this range is great enough to be of considerable significance in plant water relations. In about 80 soils which were studied (37), a minimum of 11% and a maximum of 30% of the moisture content between the moisture equivalent and the ultimate wilting point was in the wilting range.

The "readily available moisture" in a soil is that which can be used by plants in growth and is therefore the moisture above the permanent wilting percentage. While gravitational water is readily available to plants, it usually drains off too soon to be of much importance. The readily available water is therefore usually considered to be that included in the range from field capacity or moisture equivalent down to the permanent wilting percentage. In sandy soils this range is quite narrow, in clay it is quite wide. The advantages of a wide range of available water in carrying plants through droughts or in obviating the need for frequent irrigation is too obvious to need discussion. The relative availability of water in the upper and lower part of this range will be discussed later. As previously stated, plants can absorb water from soils drier than the permanent wilting percentage, but absorption is too slow for growth.

"Relative wetness" is a term applied to the ratio of moisture content to moisture equivalent (23). Dividing the moisture content by the moisture equivalent enables comparisons to be made between soils or soil horizons which differ in texture. This is particularly useful in following moisture changes at various depths or in various parts of an orchard or field where the soil is not uniform in texture.

The "water-supplying power" of the soil refers to the rate at which water moves from soil to an absorbing surface, such as a root. This term is generally used to refer to measurements made with the "soil point cones" of Livingston and Koketsu (59*a*).

MOVEMENT OF SOIL MOISTURE

Movement of soil moisture is relatively complex because of the various directions and states in which it moves and the various forces operating to cause its movement. Downward movement occurs when the soil is being wetted by rain or irrigation, some upward movement occurs when the surface is drying by evaporation, and lateral movement also occurs. Water moves as liquid in capillary films and in the larger or non-capillary pores. Appreciable movement also occurs in the form of vapor along vapor pressure gradients and in convection currents of the soil atmosphere. The forces causing movement of liquid water are chiefly gravity, hydrostatic pressure and capillary forces. Because it is often difficult to determine precisely which forces are bringing about water movement, it is considered best to regard water as moving along gradients of decreasing free energy, a statement that is true regardless of the nature of the forces involved.

Infiltration. Infiltration of water into the surface is the first step in wetting a soil, and the rate of infiltration into a given soil is a very important factor in determining how much of a given rainfall will be accumulated in the soil and how much will be lost by run-off. A relatively impermeable surface is developed on a bare soil surface after only a few minutes' exposure to rainfall. This results from the packing of small particles around the larger ones by rain drops and surface flow so that water cannot penetrate freely. Formation of such a layer can be avoided and run-off can be greatly decreased by mulches and incorporation of organic matter into the surface of the soil. According to Duley (28), formation of an impermeable surface layer has more effect on infiltration of water into Nebraska soils than soil type, slope, moisture content or profile. Run-off and accompanying erosion, in his opinion, can be practically eliminated by maintaining a mulch of crop residues on the soil. While infiltration into a bare soil is much more rapid at first if it has been cultivated, the rate decreases very rapidly after the first 15 to 30 minutes, and on both cultivated and uncultivated soils it soon reaches a constant rate which is determined by the rate of downward percolation through the deeper soil horizons.

Movement of gravitational water. This movement after the water has penetrated into the soil is chiefly affected by number, size and continuity of the air spaces or non-capillary pores through which it percolates. It usually moves very freely through the large pores of sandy soils, and such soils are generally quickly drained to field capacity. Movement is less rapid through clay because the pores are much smaller; they are frequently blocked by the swelling of colloidal gels, and air is often trapped in them. Lutz (61) found the permeability of clay to decrease as the hydration of the particles increased. Movement of gravitational water is frequently hindered by impermeable subsoil layers which trap air as well as water. Movement is facilitated by worms and other animal activity and by decay of roots, all of which leave passageways. In general, unless a hardpan interferes or the soil is saturated to a shallow water table, gravitational water can be expected to drain out of the surface layer within two or three days after rain or irrigation.

Movement of capillary water. The earlier discussions of the movement of soil moisture were based on a relatively simple concept of the soil as an aggregate of capillary tubes of various dimensions, and many present-day discussions make use of this capillary theory. According to this theory, as developed by Briggs (12), soil moisture exists principally as continuous thin films around the soil particles and in the smaller spaces and angles between them. These films are under great inward pressure because of the surface tension of the water, and water therefore tends to move from regions with thicker films to regions with thinner films.

While the foregoing assumptions are correct, the capillary theory has been found inadequate to explain certain observed facts and has therefore been sharply criticized. Dissatisfaction with the inadequacy of the capillary theory led to the gradual development of a theory based on the energy relations or work done during the movement of water. Buckingham (17) suggested that movement of water through the soil might be compared to the movement of heat or electricity through a conductor. He considered the driving force to be the difference in attraction for water between two portions of soil having different moisture contents and proposed the term "capillary potential" for the force required to move a unit mass of water from a unit mass of soil. This theory was further developed by various other workers (7, 32, 39, 52, 75, 83) to whom the reader is referred for more detailed discussions of various aspects of the theory.

According to certain writers (7, 97), the most important implication of the potential theory of soil moisture is that there are no such sharp boundaries or differences between various types of soil moisture as are indicated by the terms "gravitational", "capillary" and "hygroscopic water". Various methods of measuring the potential or force with which water is held by soil at various moisture contents agree in indicating that the potential is directly related to the moisture content. When the potential is plotted against decreasing soil moisture it forms a smooth curve, indicating that there is no real change in the state of water as the moisture content decreases from the field capacity past the permanent wilting percentage down to an oven-dry condition, but merely an increase in the energy required to move it. The permanent wilting percentage falls in the region of greatest curvature of the curve for potential over soil moisture, while the field capacity falls in the region where it becomes almost flat.

It has been proposed (31, 32) that the thermodynamic concepts of free energy be applied to discussions of water movement in the soil and through the plant. Such a treatment of soil moisture movement is based on sound principles and is very useful to the soil physicist, but unfortunately many plant scientists are not sufficiently familiar with the mathematical methods used to fully understand such a treatment. It is more intelligible to most workers and for most purposes it is just as satisfactory to discuss the movement of moisture in the more familiar terms of gradients of diffusion pressure, vapor pressure or diffusion pressure deficit (66) which are also based on energy relations. The plant scientist is primarily concerned with understanding the factors which affect the availability of water and its movement from soil to plant roots. Movement of soil moisture can be discussed in conventional terms if we remember that regardless of the terminology its movement is determined by differences in energy or in the forces with which it is held in different regions of the soil. Using this conventional terminology, we may say that water flows under the influence of gravity, moves in capillary films and diffuses as vapor, but it always moves along a gradient of decreasing free energy. Its energy is highest in free water, lower in moist soil and still lower in dry soil. Movement of capillary water is materially affected by soil texture, being most rapid in sandy soils and slowest in clay soils at saturation, but in drier soils the effects of texture are reversed, movement being slowest in sands and most rapid in clay (70). Height of capillary rise also depends on texture, being greatest in clays and least in sands. In no instance, however, has capillary rise been found to be as great as would be expected from calculations based on size of soil particles. Neither has the movement of capillary water proved to be as rapid as it was once supposed to be. Early discussions of this subject gave the impression that as rapidly as water is removed from the soil particles in contact with the roots it is replaced by capillary movement from more distant soil particles. More recent investigations indicate that capillary movement of soil moisture from moist to drier regions is very slow except where the water table is within three or four feet of the surface. Of course, some movement always occurs from regions with thicker films to regions with thinner films, provided continuity of films exists, but such movement is much more rapid in saturated soil than in dry soil. Moore (70) found very little unsaturated flow of moisture in soils at or below the moisture equivalent. Veihmeyer and Hendrickson (99) placed a mass of soil wet to field capacity in a large cylinder with dry soil on each side of it. After 139 days water had moved only eight inches into the dry soil.

Since capillary movement is so slow in soils drier than field capacity it is probable that during periods of rapid transpiration the available water on soil particles in contact with the roots is removed much faster than it can be replaced by capillary movement. Thus each absorbing root may become surrounded by a slender cylinder of soil from which all available water has been removed, although soil a few millimeters away is still at field capacity. Data of Richards (76), however, indicate that water movement possibly may occur fairly rapidly over distances of a few millimeters. He found that a pressure of 16 atmospheres reduced the moisture content of a soil layer five to ten millimeters in thickness from saturation to below the permanent wilting percentage in 24 to 36 hours. In his experiments he displaced water from a moist soil through a collodion membrane, but did not cause it to move from moist soil into dry soil.

Movement of water vapor. As the soil dries out the water films become discontinuous and capillary movement ceases. Any water movement in air-dry soils must be in the form of vapor. According to Lebedeff (57), in soil above its hygroscopic coefficient the atmosphere is normally saturated. Under field conditions, therefore, the soil atmosphere is always saturated, except the surface layer which occasionally becomes air-dry. Movement of water vapor is along vapor pressure gradients; hence it is affected by the relative temperatures and vapor pressures of various horizons of the soil and of the soil and air. Lebedeff states that the movement of water in the form of vapor is of considerable importance, especially in southern Russia and other semi-arid regions where there is no direct connection between the water table and the capillary water in the upper layer. Film movement is exceedingly slow under such conditions, so the effects of movement of water vapor are more noticeable. Lebedeff found that in winter appreciable quantities of water move from warmer, deeper levels to the cooler surface where it condenses, the amount so moving in one winter amounting to 66 mm. During a cool period in summer or autumn when the surface layer is cooled, water moves from the deeper levels to the surface whence it evaporates during warmer periods, thus slowly drying out the deeper layers. Ordinarily the surface layer of the soil is warmest during summer, and presumably some water vapor then diffuses downward where it condenses in the cooler soil, forming liquid water. According to Lebedeff, this is an important source of ground water in southern Russia. During the night the surface layer becomes cooler than the soil a few centimeters below the surface, while the reverse is ordinarily true during the day. These diurnal variations in soil temperature produce variations in vapor pressure which result in diurnal variations in water movement. Lebedeff calculated that in the vicinity of Odessa over 70 mm. of water are condensed in the surface layer of soil annually during periods when it is cooler than the air above it.

Evaporation. The quantity of water lost from soil by evaporation has been the subject of considerable controversy. The amount of water vapor lost depends primarily on the steepness of the vapor pressure gradient from soil to air which in turn depends on both soil and atmospheric factors. The vapor pressure of the atmosphere is affected chiefly by the humidity of the air. Air movement is also important because it changes the air in contact with the soil surface, preventing it from becoming saturated. The principal soil factors affecting evaporation are temperature and moisture content. Differences in evaporation from dark- and light-colored soils and from north- and south-facing slopes result from differences in temperature.

It is obvious that evaporation from a dry soil surface will be much less than from a moist one because diffusion of water vapor through the soil is very slow. Since when no rainfall occurs the only way the soil surface can be kept moist is by upward capillary movement of water, it has long been assumed that prevention of capillary movement by cultivation will greatly reduce loss by evaporation. Experiments by King (53) showed that less than half as much water was lost from soil covered with a loose dry surface layer two or three inches deep as from an undisturbed surface. King's experiments were with columns of soils in contact with free water. According to Baver (7), Eser had shown in 1884 that evaporation from soil in contact with free water is two to four times as fast as from well drained soils. Unfortunately most people failed to realize that evaporation from soil in contact with a water table occurs much more rapidly than evaporation from soil that does not have a water table near the surface. As a result of this misunderstanding the advantages of a dust mulch in agricultural practice were greatly overemphasized.

More recent experimental work has shown that evaporational losses are less than commonly supposed and that they are not much reduced by cultivation. This is primarily because the water table is so far below the surface in most cultivated land that little upward movement to the surface can occur. Considerable experimental evidence is available which indicates that if the water table is even a few feet below the surface little upward movement of water occurs. Shaw and Smith (89) found considerable water movement to the surface of Yolo sandy loam and Yolo loam with a water table four feet below the surface, but very little when it was ten feet below the surface. They concluded that no appreciable upward movement of water to replace loss by evaporation occurs in these soils when the water table is ten or more feet below the surface. Other investigators have found evidence of slow or negligible capillary rise where the water table is more than a few feet below the surface (18, 52, 80, 96). Where rainfall seldom or never wets the soil to the water table, as in much of the plains area, upward movement is probably negligible. In such soils removal of water by transpiring plants is a much more important factor in drying out the soil than evaporation (22).

Veihmeyer and Hendrickson (102) cite work in Russia and in this country indicating that little water is lost by evaporation from below the first foot. It has been demonstrated (93) that under California conditions most of the water lost by evaporation comes from the upper four inches, much less from the second four inches and very little from below eight inches. By the time the surface soil has dried sufficiently to permit cultivation considerable moisture has already been lost and more is lost from the freshly stirred soil. In general, most soil appears to dry out to about the same extent and the same depth, whether cultivated or not, unless the water table is within a few feet of the surface. Cultivation may reduce loss by evaporation on soils which crack badly, but it is claimed (103) that the cracks in most California soils, including clays, are too shallow to increase water loss seriously. Cultivation of summer-fallowed land may of course be necessary to prevent a crop of weeds from removing the accumulated moisture. This problem is discussed at length by Lyon and Buckman (62, 223–227).

While dust mulches seem to be ineffective, it appears that mulches of straw, grass, leaves, paper and similar materials are usually much more effective in reducing water loss. This is partly because they shade the soil, reducing its surface temperature, and partly because they lengthen the diffusion gradient from soil to air and protect the soil surface from the drying effects of wind. Russel (82) considers mulches to be effective only in preventing drying of the surface layer because he says a layer of dry soil is a better insulator than the average mulch and also more impervious to water vapor.

The relative amounts of water removed from the soil by evaporation and by transpiration are of interest. It is generally accepted that plant transpiration is the chief means by which water is removed from soils and that transpirational losses far exceed losses by evaporation. If evaporation removes water only from the surface foot of soil the remainder of the soil moisture would remain untouched were it not for the roots of plants. Orchard soils in the East are sometimes dried to the wilting percentage to a depth of two or three feet within three weeks (64), and prune trees exhaust the readily available water in the top six feet of soil in about six weeks in midsummer at Davis, California (44). It was found (103) that mature peach trees in the Sacramento and San Joaquin valleys of California absorb the readily available water to a depth of six feet from sandy soil in about three weeks and from clay loam soil in about six weeks. Citrus fruits on sandy loam soils four to six feet deep in San Diego County require irrigation every six weeks during summer, and on more shallow soils irrigation is required more frequently. In such instances loss by transpiration is several times greater than loss by evaporation. In certain experiments (96) a tank with bare soil surface lost 18.9 pounds per square foot of surface in four years, equivalent to a depth of 3⅜ inches of water, or less than one inch per year. A four-year-old prune tree growing in a similar tank lost 1,250 pounds of water by transpiration in one growing season. An acre of deciduous fruit at Davis, California, used eight acre-inches of water in about six weeks in mid-summer, or about one pound per square foot of soil surface per day. It seems clear that plant transpiration is the chief means by which capillary water is lost from the soil, and it therefore appears that maintenance of vegetation cover on watersheds decreases the amount of water stored in the soil. This of course does not reflect on the possible importance of vegetation in reducing erosion and consequently preventing silting up of reservoirs. Kittredge (54) stated that a forest transpires more water than would be lost by evaporation from the same area, hence more water could be obtained from a bare watershed than from a forested one. He recognized the need for plant cover to control erosion and slow down run-off and suggested that species with low transpiration rates be selected, recommending scrub oaks and grasses for California.

AVAILABILITY OF SOIL MOISTURE TO PLANTS

The gravitational water occurring in saturated soils is readily available to plants but is seldom present long enough to contribute much to plant growth. If it does remain more than a day or two its injurious effects overshadow any benefits resulting from its availability. For most plants, then, the water readily available for growth is the capillary water in the range between the field capacity and the permanent wilting percentage. The best moisture supply for growing plants is afforded by soils in which this range of available water is wide. Soils show great variations in this respect, but sandy soils generally have narrow ranges of available water and clay soils wide ranges. Among some soils used by the writer (56) was a pure sand containing only 2%, a sandy loam containing 14% and a clay containing 19% of readily available water. Occasionally soils with high moisture equivalents and field capacities also have very high wilting percentages and contain but little readily available water. For example, in a California study (104), a clay was found to have a moisture equivalent of 31% and a wilting percentage of 25%; it therefore contained only 6% of available water or less than many sandy soils.

Plants growing in soils with a low storage capacity will exhaust the readily available water and suffer from desiccation much sooner than plants growing in soils with a very large storage capacity. Where irrigation is practiced the more frequent applications required result in much greater waste of water by run-off and evaporation than where a few irrigations suffice. This is especially important with shallow-rooted crops, and Veihmeyer and Hendrickson (104) cite several examples of such occurrences.

Much discussion has occurred as to whether water is equally available over the entire range from field capacity to wilting percentage. Veihmeyer and Hendrickson have repeatedly stated that water either is available or is not available to plants, and that it is equally available over the entire range from field capacity down to the wilting point where it becomes unavailable for growth. They have reported results of experiments indicating that the growth and quality of apples and pears (47), grapes (43), peaches (42), prunes (44), walnuts (45) and cotton (1) were not affected by the moisture content of the soil unless it fell to the wilting percentage and remained there for some days. These plants did no better on frequently irrigated plots than on plots where the soil moisture was allowed to fall to the permanent wilting percentage before water was applied. The seeds of many species are reported to germinate equally well over the entire range of moisture content from wilting percentage to field capacity (27). A few, however, germinated better at 1% or 2% above field capacity, and celery seed was found not to germinate at all in the lower range of soil moisture.

The apparent equal availability of water over the entire range is explained on the basis that there is but a small change in the forces with which water is held by the soil over the range from field capacity to permanent wilting percentage. The permanent wilting percentage occurs at the moisture content where these forces begin to increase very rapidly, and a small decrease in soil moisture therefore is accompanied by a very rapid increase in the force required to move water from soil to roots.

Considerable evidence is available indicating that water is not equally available to plants over the entire range from field capacity down to permanent wilting. The growth rate of apples in Maryland orchards was reduced when the driest part of the root zone approached the permanent wilting percentage, which was long before the entire root zone was dried to that point (64). Stomatal behavior of apples (64) and peaches (50) was also affected by soil moisture, the stomates being open for a shorter period each day in dry than in moist soil. Premature stomatal closure presumably reduces photosynthesis, resulting in a deficiency of carbohydrates. It was found that in very heavy soils in Oregon the rate of growth of pears is closely related to the moisture content of the upper three feet of soil (2, 58). The fruits suffered reduction in size when the soil moisture dropped below about 70% of the readily available moisture. Trees in these soils have very uneven root distribution, and it may be that while the soil in contact with the roots was at the permanent wilting percentage considerable volumes of soil not penetrated by roots were left at field capacity. As a result the average moisture content would appear to be above the wilting percentage, although the moisture content of the soil in which the roots were growing was actually reduced to the wilting percentage. The transpiration rate of loblolly and shortleaf pine seedlings grown in containers decreased with decreasing moisture content before the permanent wilting percentage of the soil was reached (86). This, likewise, might have resulted from uneven absorption of water because of uneven distribution of root systems. Furr and Taylor (38) found that lemons on shallow soil underlain by dense subsoil suffered sufficient water deficit to cause reduction in size of fruit before the moisture content of all the soil in the top foot was reduced to the permanent wilting percentage. They suggested that some discrepancies in conclusions regarding the availability of water result from differences in judgment as to what constitutes permanent wilting. In a heavy clay soil with a field capacity of 33% the basal pair of leaves of well established sunflower plants wilted at 20.2%, but the entire plants did not wilt until the soil moisture was lowered to 16.2%.

The growth rate of sunflower plants was affected by small differences in soil moisture content, even though the moisture content was never allowed to fall to the permanent wilting percentage (65). Growth of young maize plants was also slowed by decreasing soil moisture and ceased before the soil moisture content fell to the permanent wilting percentage. Water appeared to be less available for growth from a moisture content 2% or 3% below capillary capacity, and growth ceased while 3% of available water remained in the soil (25). Growth of *Cyperus rotundus* in pots also appeared to be checked by decreasing availability of water in soil which was always above the wilting percentage (26). Each decrease in the minimum soil moisture percentage reached before rewetting to saturation resulted in a significant decrease in weight of the tops of nut grass. Tuber development was decreased significantly by decreased soil moisture from a moisture content only 2% below the moisture equivalent. The growth and yield of kidney beans were reduced if the soil was allowed to dry part way down to the permanent wilting percentage before watering, even though the moisture content never actually reached the permanent wilting percentage (6).

It was found that a decrease in soil moisture from field capacity to the permanent wilting percentage (first permanent wilting of basal leaves) caused the osmotic pressure of sunflower plants in dry air to increase about five atmospheres and that of plants in moist air to increase about two and one-half atmospheres (37). From these and other data the investigators concluded that plants are subjected to progressively increasing water deficit from a moisture content about half way between the moisture equivalent and the permanent wilting percentage down to the permanent wilting percentage. This is in accord with the observation of the writer (56) that exudation from the stumps of detopped plants ceases while about 45% of the soil moisture available to intact plants is still present. This situation probably results from the fact that the roots alone cannot absorb water against a potential of more than one or two atmospheres, but when attached to a transpiring shoot they can absorb against a potential of several atmospheres. Intact plants can therefore absorb water from much drier soil than can isolated root systems.

The contradictory opinions concerning the availability of water held by various investigators results at least partly from differences in the soil types used, differences in opinion as to what constitutes permanent wilting and differences in interpretation of the data. Where the soil is thoroughly and uniformly permeated by roots it is likely that plants can reduce the average moisture content much nearer the permanent wilting percentage without suffering from a water deficit than in heavy soils where root systems are scanty and unevenly distributed. There is no doubt that more energy is required to move water from soil to roots in a dry soil than in a moist soil. This may not immediately decrease transpiration or growth because as the diffusion pressure deficit of the soil increases, the osmotic pressure and diffusion pressure deficit of the plant may at first increase proportionately. Thus the same gradient from soil to root is maintained, while the increased osmotic pressure of the plant sap does not materially reduce transpiration (36). There is, however, an abundance of data indicating that as the soil moisture decreases to the lower half of the range of readily available water, growth and yield are often decreased before the permanent wilting point is reached.

Concentrataion of soil solution. Another factor affecting the availability of soil moisture is the concentration of the soil solution. It is recognized that high soil concentration may hinder plant growth by toxic effects of the ions as well as by their osmotic effects, but the latter often seem to be quite important. Addition of NaCl producing an osmotic pressure of four atmospheres caused severe wilting of tomato plants (60). In other experiments high solute concentration increased hydrostatic stress and seriously checked growth of tomatoes (41) and kidney beans (6). It was reported from California (63) that normally fertile irrigated soils had a soil solute concentration at the permanent wilting percentage of 1.3 to 1.8 atmospheres. Some soils with osmotic pressures at the permanent wilting percentage of two to four atmospheres produced good crops of alfalfa, cotton and wheat, but those with higher values showed reduced yields, and soils with values of 40 atmospheres or higher were barren.

Soil temperature also affects the availability of water to plants. Many years ago Livingston suggested that temperatures probably markedly influenced the water-supplying power of soils (73). The writer (55) found that the water-supplying power at 0° C. was only about half as great as at 25° C. The decreased rate of movement from soil to absorbing surface results principally from the increased viscosity of water at low temperatures.

MEASUREMENT OF SOIL MOISTURE

Since a review of this subject by another author is expected to appear in this journal the measurement of soil moisture will be discussed very briefly. Two types of methods are used, those giving the actual moisture content and those measuring the forces with which moisture is held or the rate at which it is supplied to an absorbing surface. Livingston and his co-workers (73, 107) have frequently stated that the capacity of the soil to supply water to roots is the essential factor of soil moisture as related to plant growth, and all other factors such as texture, water-holding capacity and permanent wilting percentage are important only as they affect the water-supplying capacity of the soil. This view led to the development of soil point cones to measure the water-supplying power of the soil (59*a*).

The actual moisture content of the soil is usually expressed as a percentage of the oven-dry weight, but it could perhaps be expressed more advantageously on a volume basis. The primary interest is in the volume of water available to the roots occupying a given volume of soil, rather than the weight of water in a given weight of soil (7). Various methods have been developed to determine soil moisture without the delay required by oven-drying and without the disturbance caused by sampling. Attempts have been made to determine soil moisture electrically by measuring the electrical conductivity (9), electrical capacity (4, 5), dielectric constant (35) and heat conductivity (48, 88). Physical methods include use of tensiometers (77, 78, 98), porcelain soil point cones (59*a*), and devices to measure the pressure required to penetrate a soil mass (3). Several of these methods can be used to measure moisture content in the root zone without disturbing the roots, a procedure likely to yield much useful information.

EXPERIMENTAL CONTROL OF SOIL MOISTURE

The older literature dealing with soil moisture as a factor in plant growth frequently described experiments in which plants were said to have been grown in containers maintained at certain definite moisture contents, as at 10%, 15%, 20% and 25% of the dry weight of the soil. Other papers have mentioned maintenance of soil moisture at optimum, sub-optimum and supra-optimum contents. Engineers have also described methods of wetting soil to be used in construction to some predetermined moisture content by sprinkling a certain amount of water over the surface.

The impossibility of doing these things should have been realized by all who have observed the distribution of moisture in the soil after a rain. Strange to say, however, it was not until comparatively recently that Shantz (87) and Veihmeyer (93) called attention to the fact that if a small quantity of water is applied to a mass of dry soil the upper layer is wetted to the field capacity and the rest of the soil mass is completely unaffected. Addition of more water results in wetting the soil to a greater depth, but there will always be a sharp line of demarcation between the wetted and unwetted soil. This situation has been observed by everyone who has dug in soil following a summer shower and observed the sharp line of demarcation between the wet soil and the dry soil beneath. Since the field capacity is the amount of moisture held against gravity it is obviously impossible to wet any soil mass to a moisture content less than its field capacity. If a container is filled with dry soil having a field capacity of 30% and enough water is added to wet the whole mass to 15%, one half of the soil will be wetted to field capacity and the other half will remain dry. Obviously the earlier investigators did not really maintain their plants at the specified soil moisture contents, but merely gave them various amounts of water distributed in various proportions of the soil mass. The difficulties of controlling soil mosture have recently been discussed (46).

Numerous investigators have attempted to devise means of controlling the moisture supply and improving its distribution in the soil. Livingston (59) developed the use of porous porcelain cups buried in the soil and connected to a reservoir as a means of controlling the supply of moisture, and this system later became known as the auto-irrigation system. He attempted to limit the water supply by introducing mercury columns of various heights between the irrigator cone and the reservoir, thus increasing the tension necessary to bring about water movement from reservoir to soil. Unfortunately there is a tendency for roots to become massed around the irrigator cones, somewhat nullifying the control, as they absorb directly from the surface of the cones rather than from soil which has come into equilibrium with them. An improvement in moisture distribution is afforded by the use of double-walled pots with a space for water between the glazed outer wall and the porous inner wall (107). The high cost of these containers has prevented their extensive use. Richards and Loomis (79) found double-walled pots maintained a constant soil moisture content for small plants with low tensions (short mercury columns) but not for large plants which removed water rapidly. Even with tensions as low as two to four centimeters of mercury, water was removed faster than it could be supplied. This is because water moves so slowly from wet to dry soil. Numerous tests indicate that many greenhouse plants grow better when supplied with water by some type of auto-irrigator than when watered manually. Use of short pieces of glass rope to supply water to potted plants has recently been described. One end of the rope is pulled through the hole in the bottom of a pot and spread out in contact with the soil mass, while the other end dips into a reservoir. Such devices are particularly useful for house plants which usually are over or under watered. They have also been successfully applied to watering seed flats and greenhouse benches (71).

A number of methods of controlling soil moisture content have been described (8, 33, 49, 67, 69), but none is entirely successful. It seems probable that there is no satisfactory method of constantly or permanently maintaining any soil at a moisture content below its field capacity. Plants growing in the soil may be allowed to reduce the soil moisture to any desired level between field capacity and permanent wilting percentage before applying water, but the moisture content cannot be maintained at any constant level within this range. Most of the proposed control methods simply reduce the amount of water per plant by reducing the soil mass which is wetted, and none of them permanently maintains the entire mass of soil at a uniform moisture content below its field capacity. Discussions of the "optimum" soil moisture content are therefore largely academic and have little relation to actual field conditions. Soil moisture content as related to plant growth can be evaluated only in terms of its relation to the field capacity and the permanent wilting percentage of the soil in which the plants are growing.

SUMMARY

The soil is a complex system consisting of four principal components: mineral materials, organic matter, water and solutes comprising the soil solution, and air. The characteristics of a soil depend chiefly on the texture or size of the mineral particles, ranging in order of decreasing size from sand through loam to clay; on the structure or manner in which these particles are arranged, which determines the number and size of pores; and on the amount of organic matter incorporated with the mineral matter.

Soil moisture is commonly classified as gravitational, capillary and hygroscopic water and as water vapor. Capillary water, which occurs as films around the particles and in angles and the smaller pores between them, usually is the only form of moisture available to plants. There are no definite boundaries between these various types of soil moisture, and they are to be regarded as convenient but wholly artificial categories.

In a soil thoroughly wetted by rain or irrigation the gravitational water usually drains away within one or two days leaving the moisture content at the "field capacity". The field capacity usually approximates the value known as the "moisture equivalent", which is the moisture content of a soil that has been exposed to a centrifugal force of 1000 times gravity. The "permanent wilting percentage" is the moisture content of a soil at which permanent wilting of plants growing therein first becomes apparent, because water no longer moves from soil to roots fast enough to replace the losses by transpiration. That portion of the soil water which is readily available for growth lies between the field capacity and the permanent wilting percentage. The moisture content at the permanent wilting percentage depends on the soil texture, being lowest in sandy soils and highest in clay soils. Obviously the wider the range between the field capacity and the permanent wilting percentage the more water is available for plant growth.

It is sometimes claimed that water is equally available to plants over the entire range from field capacity to permanent wilting percentage, but there is considerable evidence that water becomes progressively less available in the lower part of this range.

Movement of soil water is caused principally by gravity, hydrostatic pressure and capillary forces, the last resulting from differences in curvature and thickness of water films. Since it is often difficult to distinguish between these forces it is convenient to speak of the movement of water along a gradient of decreasing free energy. The work done in moving water against the attractive forces of the soil increases with decreasing soil moisture. It is often measured in terms of height of an equivalent column of water or mercury and designated as the "capillary potential" or "pressure potential".

Movement of capillary water in a soil mass below the field capacity and lying more than a few feet above a water table is extremely slow. It is therefore likely that relatively little water moves toward the roots and that most soil moisture becomes available only as roots come in contact with it as a result of their elongation through the soil. It has been demonstrated that little or no water is lost by evaporation from below the surface 8 to 12 inches, and dust mulches are therefore relatively ineffective in reducing losses by evaporation.

Soil moisture is usually expressed as a percentage of the oven-dry weight of the soil. It would be more satisfactory if it were expressed as a percentage of its volume, but such determinations are difficult. The moisture content of soil can be measured in place by determinations of electrical conductivity and capacitance, of dielectric constant and of heat conductivity, and indirectly by use of soil point cones and tensiometers.

Numerous attempts have been made to grow plants at various moisture contents between field capacity and permanent wilting percentage. It is impossible to half wet a soil, however, and it appears practically impossible to permanently maintain any intermediate moisture contents. If insufficient water is added to a soil mass to wet it all to the field capacity, a part of it will be wetted to the field capacity and the remainder will remain unaffected.

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THE PHYSIOLOGY OF DECIDUOUS FRUITS IN STORAGE

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INTRODUCTION

The various deciduous fruits like apples, pears, peaches and plums are living organisms, even after being separated from the parent tree at harvest time. This means that these fruits carry on physiological processes like respiration and transpiration during storage. Of course, they finally "die", and it is the job of the owner of the fruit to dispose of them long before death is reached. Just when an apple is "dead" is not easily discernible. It is true that parasitic fungi are often the cause of death, but very often old age is the cause of the "demise".

It is the obligation of the storage operator to so manipulate conditions in storage that the longest possible life of the fruit may be expected. That means he must provide conditions for controlled respiration, transpiration and possibly other physiological processes. It is the obligation of the plant physiologist to tell the storage operator what the optimum conditions for storage are.

This review purports to summarize the available information on the physiology of fruits and how it may be controlled in storage. The behavior of fruit in storage is often strongly affected by conditions in the orchard during the growing season or by the handling of the fruit by the owner after harvest. Since these pre-storage factors have a bearing on storage behavior, they will be dealt with whenever they seem pertinent.

TRANSPIRATION

All deciduous fruits should reach the consumer while they are still crisp and juicy. Most of these fruits are 85% or more water, and even a comparatively small loss in total water content means an appreciable reduction in eating quality. By the time an apple fruit has lost 5% of its original weight, it is shrivelled enough that it not only has reduced eating quality but is unattractive because of the wrinkled appearance of the skin.

Effect of Time of Harvest. It has been a common observation that deciduous fruits picked in a rather immature condition shrivel more in storage than fruits picked at the proper time. For example, if Golden Delicious apples are examined in storage on February first, the fruits that were harvested on September first will show more wilting than fruits harvested October first. Because of this observation it has been assumed that early picked fruits transpire faster in storage than more mature ones.

Recent work (150) throws light on this subject with reference to apples. Whether these findings will be corroborated by other deciduous fruits remains to be seen. It was found that the rate of transpiration of apples does tend to be very high early in the growing season, but as the season progresses the transpiration rate decreases until about the normal time for harvest. The transpiration rates of several varieties of apples were found to reach their minimum shortly before the time for optimum picking maturity. After this point was reached, the apples transpired at a faster rate, especially when allowed to become over-mature on the tree. Hence it seems apparent that late picked fruits may actually transpire at a faster rate than earlier picked fruits in storage.

The fact that Golden Delicious apples examined on February first show more shrivelling if harvested early may be explained in the following ways. First, they are in storage a month longer, so it is no great wonder that the total water loss is greater. Second, during the early storage period in most cold storages, the relative humidity is likely to be rather low. This low humidity may be due to the very low refrigerant temperatures employed. It might also be due to the drying effect of the wooden containers until the vapor pressure of the wood comes to equilibrium with that of the air. Third, the temperature of these storages and of the fruit is often not so low as it is later in the season when the second lot of Golden Delicious is placed in storage.

The respiration rate of apples and pears varies with the age of the fruit, but no correlation was found between respiration rate and transpiration rate (149). As fruits grow old the rate of transpiration eventually diminishes. This is probably due to differences in the physical structure of the skin and to internal factors (170).

Effect of Fruit Size. Since transpiration is a surface phenomenon it is not surprising that a bushel of small apples will transpire at a faster rate than a bushel of large apples. It has been found (149) that the transpiration rate of several varieties of apples in storage was directly proportional to the surface area of the fruit. It is recommended that transpiration results be expressed on a surface area basis rather than on a fruit weight basis as they usually are (149).

Effect of Physical Nature of the Skin of the Fruit. It is obvious that transpiration from fruits is unlike evaporation from a free water surface if for no other reason than that the skin of the fruit inhibits water loss. Just how much the skin of different varieties and the skin of fruit of different maturities within the same variety affects transpiration rate is not entirely clear.

It is commonly observed in the literature (26, 146) that the thickness of the skin and of the cuticle markedly affects the rate of transpiration. This is based on the observation that a variety like Golden Delicious shrivels badly in storage and that it has a thin cuticle. That the skin inhibits the transpiration rate is inescapable. It has been shown that a unit area of free water surface will evaporate 70 times more water than a unit area of the uninjured fruit of an apple per unit of time (169).

It is also true that when the waxy layer on the surface of fruits is removed by brushing or by washing with severe treatments (like sodium silicate washing), the rate of shrivelling is greatly increased (131). The importance of the nature of the surface of the fruit as it affects transpiration is also demonstrated by the effect of wax additions to the fruit in checking water loss (173).

Careful studies made on cuticle thickness and transpiration rate in apples have not always shown a good correlation (149). While it has been found that Golden Delicious did shrivel badly and also had a thin cuticle, this correlation did not hold for other varieties necessarily. Baldwin and Rhode Island Greening have the same cuticle thickness, on the average, but they transpire at different rates (149). It would seem, then, that other internal factors are sometimes more effective in determining transpiration differences.

Other skin factors that may affect transpiration rate are number and size of lenticels in the skin. It has been shown, however, that about 70% of apple transpiration is cuticular and only 30% lenticular (149). Cell wall thickness and arrangement of cells in the skin may also be factors in determining transpiration differences between varieties (26).

Effect of Water Vapor Pressure Deficit. The fundamental reason why fruits lose water vapor in transpiration is that there is a difference in the water vapor pressure of the fruits' internal atmosphere and in that of the surrounding atmosphere. The relative humidity of the internal atmosphere of fruits is presumably 100% under normal circumstances. Of course, after the fruits become partially desiccated, it is probably not that high. Hence if apples are held in atmospheres with a relative humidity less than 100%, water vapor is likely to move from the fruit to the surrounding atmosphere (166, 168, 171). Then, ideally, all deciduous fruits should be stored in a relative humidity of 100%. The usual recommended figure is from 85% to 90%. This recommended figure is a compromise between the ideal and the practical. If fruits are held in atmospheres saturated with water vapor, surface molds are very likely to grow on them and on their containers. These surface molds do not directly affect the fruits. The musty odor emanated by the molds is often absorbed by the fruits and contributes towards a musty flavor in them. The presence of these molds on the fruits and containers is unsightly. With a relative humidity of even 85% or 90% there is often a growth of these surface molds or "whiskers". This mold growth probably is due to the fact that the dewpoint of the fruit or containers is reached at times and there is condensation of moisture in a thin film. It is in this moist film that the molds grow. If humidities as high as 90% or 95% are used, some provision must be made for mold control. Use of ozone seems to offer some promise as a means of mold reduction in these very high humidities (198).

Relative humidity is not the only storage factor that determines the rate of water loss from fruit. Transpiration may be more rapid in a lot of fruit at 85% relative humidity at 36° F. than it is in a similar lot held at the same humidity in 32° F. air (66). This is due to the temperature factor. As the temperature rises the vapor pressure of water rises, and the difference (or deficit) between fruit and atmosphere vapor pressures is increased. Another illustration of this temperature effect is encountered when warm fruit is placed in a cold room. Let us assume that a lot of apples at 73° F. is moved into a cold storage room at 32° F. which has a relative humidity of 100%. Transpiration is relatively rapid until the fruit temperature reaches the air temperature. The water vapor pressure of the internal atmosphere of the apples (at 73° F.) is 23.8 millimeters of mercury in this hypothetical case. The water vapor pressure of the atmosphere of the cold storage room (note that relative humidity is 100% in both cases) is only 4.6 millimeters of mercury. There is, hence, a difference or deficit of 19.2 millimeters of mercury. This deficit is about eight times as great as the theoretical deficit occurring in a situation where apples, with an internal atmosphere of 100% relative humidity, are held in air at 32° F. with a relative humidity of 50%. The most striking illustration of this effect of temperature difference, as it affects vapor pressure deficit, is seen when a rapidly transpiring plant product like lettuce is moved into storage. Lettuce removed from the field at a high temperature and placed in a 32° F. storage with a relative humidity of 100% will sometimes show wilting. Apples do not transpire rapidly enough to show this wilting, but certainly the effect on transpiration is measurable. The only apparent practical solution to this problem is to accomplish rapid cooling after fruits are placed in storage. That is, the faster the differential between room and fruit temperature is eliminated the less will be the transpiration as a result of water vapor deficit.

The foregoing discussion was based on a hypothetical situation, but actual investigation shows the soundness of the theory. It has been shown that transpiration of apples is directly proportional to the water vapor deficit in any situation (149)[1].

While more detailed studies have been conducted with apples than with any other deciduous fruit in this connection, the general effects of vapor pressure differences seem to apply to other fruits (5, 167).

There is another temperature effect on transpiration that may affect the locus of transpiration. It has been shown that if one side of an apple is colder than the other in storage, there will be a distillation of water from the warm side to the cold side, leaving the warm side withered (27). This may occur when one side of a fruit is cooled much more rapidly than the other.

Effect of Air Movement. It would seem logical to suppose that transpiration would be accelerated by air currents passing over fruit in storage. The magnitude of this effect is of commercial interest because some storages operate with forced air circulation, others only with natural convection currents.

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[1] Eventually the time factor will upset this straight line relationship between vapor pressure deficit and transpiration. That is, in time skin structure may change enough with large water deficits to slow down the rate of transpiration.

Several studies have shown accelerated transpiration rate of 30% to 100% when air currents of varying velocities were used with apples and grapes (5, 164). Some of these experiments are open to criticism in that the exact relative humidity around the fruits was not measured during the trials. The author has found that when fruits are subjected to different air flow rates in closed containers it is difficult to maintain the humidity at a constant level. For example, when a slow air flow rate is used, the relative humidity around the fruit is usually a little higher than the theoretical value, even though the air has been conditioned by passing it through sulphuric acid-water mixtures. Hence it is difficult to attribute differences in transpiration to velocity rates alone in studies where humidity values are not specified. Smith (165) overcame this objection by moving apples through still air at a known velocity by means of a turntable. In an early study (164) he did not specify the temperature and humidity, but in a later paper (165) on this topic he used 10° C. air at 80% relative humidity. He found increased tranpiration with apples up to a velocity of 300 centimeters per minute.

Pieniazek (149) noted that the effect of air movement on transpiration of apples was negligible, not exceeding an increase of 5%. The effect was very small when high relative humidities were used, and, of course, high humidities are recommended in cold storage practice. He used Smith's technique (165) of moving the apples through air at a known humidity.

It is sometimes claimed by storage operators that apples stored in forced air rooms show more shrivelling than apples in "still air" rooms. It has not been shown, however, that the relative humidity was identical in each case around the fruits. In the light of Pieniazek's work it would seem safe to conclude with apples, at least, that rather high velocities of air do not markedly affect transpiration if the humidity is as high as it should be. The advantages of rapid cooling with moving air would doubtless outweigh the disadvantages of slightly increased transpiration.

Practical Implications of Transpiration Studies. It would seem safe to conclude that ideally the smaller the water vapor pressure deficit between fruit and atmosphere the better. This principle should apply not only during storage but while the fruit is being displayed and while in the consumer's kitchen. Certainly one of the primary causes of loss in quality is loss of moisture. The practical limitation of using very high relative humidities lies in the fact that surface molds may be troublesome. The whole problem of control of these molds should be investigated more thoroughly by pathologists. While ozone offers some promise along this line, its use may not always be practical.

Storage at temperatures as low as is compatible for best keeping of various species and varieties of fruit is certainly logical. Prompt cooling of the fruit after picking would seem to be a logical step from the standpoint of reduced moisture loss.

Use of protective coatings may be of some value in special cases for the reduction of transpiration. Additions of wax to the skin of certain varieties may pay under some circumstances (173). Use of special wraps, such as Pliofilm, Cellophane, aluminum foil or latex, may be of some practical value in this connection (13). The precaution that must constantly be kept in mind in connection with putting any "moisture proof" wrap or coating on fruits is that they must be reasonably permeable to both oxygen and carbon dioxide (13). Otherwise off flavors and physiological disorders may occur.

RESPIRATION

No attempt will be made to discuss the nature of respiration, since that is a subject in itself (17, 18, 38, 118, 157, 180, 190). It should be emphasized, however, that when the equation $C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O +$ energy is used, it is an admitted oversimplification. There must surely be preliminary steps and side reactions involved in fruit respiration. When the full nature of fruit respiration is understood, perhaps we shall be able to do a still better job of regulating the course or rate of respiration to practical advantage. Only aerobic respiration is stressed in this review because it is of more direct interest in storage physiology. To be sure, anaerobic respiration may occur in varying degrees in fruit in storage under some circumstances.

Effect of Age of Fruit. Early workers (15, 58) on fruit respiration paid little attention to the effect of age. Burroughs (20) was among the first to recognize that the respiratory rate of apples is not constant at a given temperature. Later work in this country

(75) and in England (108) emphasized the marked effect of fruit maturity on respiration.

Kidd, in England (100), and later workers (119, 162), in America, showed the changes in respiration during ontogeny. Following fruit setting, the respiration rate of various apple varieties is very high. After the rate declines to a minimum, at about the time most apple varieties are harvested, the rate begins to rise again to a maximum. This respiratory peak has been called the "climacteric" by Kidd and West (98). To assign a climacteric or menopause to apples and pears seems a little far fetched, but the term has now been widely accepted by investigators all over the world. Following the climacteric, the respiratory rate declines in apples and pears and the fruits are said to be in "senescence" (100). Apples in storage are described as being pre-climacteric or post-climacteric, depending upon their position on the respiratory curve.

Changes in respiratory rate with age among stone fruits have not been studied so completely. Some studies (64, 134, 187) showed a respiratory rise after harvest, but the fruits were discarded because of decay before any decline was detected. At least one study (159) shows a decline after the climacteric was reached. One study with sweet cherries (55) showed no respiratory rise after harvest. None of these workers attached the significance to the climacteric in stone fruits that is often given to it in apples and pears. Peaches and plums are usually eating ripe by the time they reach the climacteric.

The magnitude of the respiratory rise in apples and pears seems to depend upon a number of factors, *e.g.*, species, variety, climatic conditions during the growing season, temperature of storage, and constitutents of the atmosphere in storage. The rate of respiration in Bartlett pears at 59° F. was seven times as great at the climacteric as it was at the pre-climacteric minimum (123). The rate of respiration at the respiratory peak in a variety of apples like Duchess of Oldenburg may be three times as great as at the minimum (175). In other varieties, *e.g.*, Jonathan (65) and Rhode Island Greening (175), the respiratory rise may be very small. Unpublished work by the author with McIntosh harvested from the same trees and stored over a three year period shows that this variety has a higher respiratory peak some years than others. In general, the respiratory rise is much more evident at high storage temperatures than at low (176). In fact, some varieties show no rise in respiratory rate at a temperature as low as 32° F., even though they are found to be post climacteric upon removal from storage (176).

Figure 1 shows a typical respiration curve for McIntosh apples at harvest time. It also shows the effect of temperature, carbon dioxide and oxygen on the respiration rate of this variety while in storage and upon removal from storage.

Not only does the total amount of carbon dioxide evolved by apples vary with age, but the respiratory quotient (amount of carbon dioxide evolved divided by oxygen consumed) also varies. The respiratory quotient of one variety of apples varied from 1.0 to 1.25 at 10° C., depending upon the age of the fruit (111).

The occurrence of a respiratory rise has some significance in the storage of apples and pears, but that significance may have been over-emphasized at times. Kidd and West (101) claimed that low temperature breakdown occurred during storage in English apples only when they were placed in storage while at the respiratory peak. A similar finding was reported for the induction of soggy breakdown (also a low temperature disorder) for Grimes Golden (74). Yet no correlation between respiration rate at the time of storage and the occurrence of soft scald in Jonathan could be found (65). Both soggy breakdown and soft scald are closely related troubles, and the latter work (65) may tend to discount the earlier work with Grimes (74).

Attempts have been made to correlate the proper time of harvest of apples to be stored with respiratory rate. Kidd and West (108) reported that some varieties should be picked just before the climacteric rise begins. Phillips (148) claimed that Canadian grown McIntosh should be harvested after the climacteric. Unpublished work by the author with this variety indicates that for long time storage in New York this variety should be picked just as the rise begins. It was found that most of the apples had abscissed from the tree by the time the climacteric was passed.

The question arises as to how accurately respiration measurements made in the laboratory reflect the true respiration rate of fruit on the tree. Ezell and Gerhardt (39) studied the respiration rate of Washington grown apples five and 24 hours after picking. When they plotted the respiratory measurements made 24 hours after harvest, they noted the usual strong climacteric rise. When

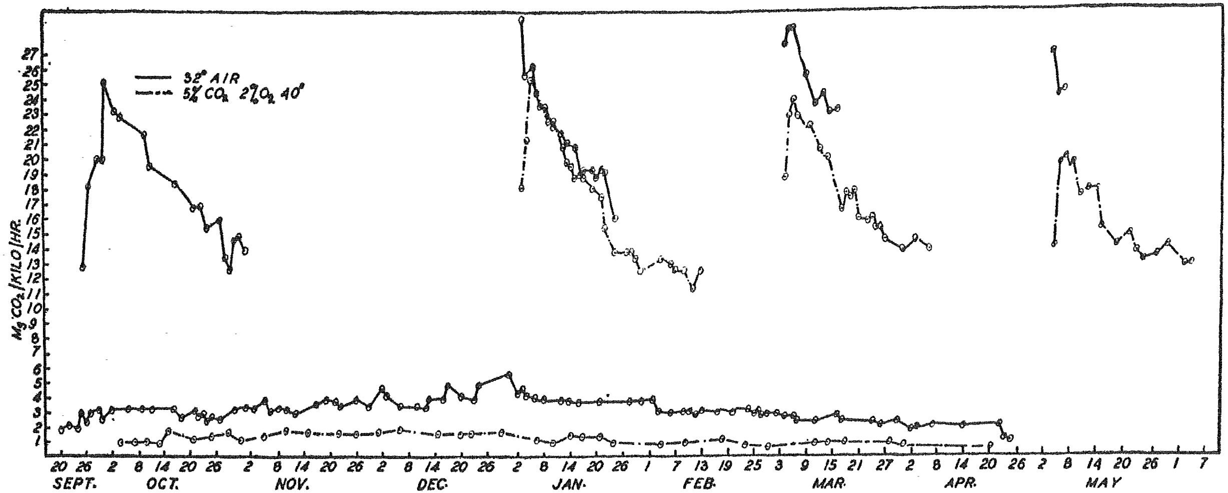

FIG. 1. Respiration of McIntosh apples in 32° F. air and 5% carbon dioxide with 2% oxygen at 40° F. (lower pair of curves). Respiration also measured at harvest time and upon removal from storage in January, March, and May (upper set of curves) at 74° F. in air (174).

measurements were made five hours after harvest on successive picking dates they did not find any such marked rise. Because they could find no sharp minimum rate prior to the respiratory rise and because they believed the usual measurements (24 hours after harvest) did not reflect the true respiratory rate on the tree, they discounted the value of this technique as an index of when to pick apples. The author believes the technique may have some value for certain varieties of apples where there is a marked minimum or a sharp rise in respiratory rate. Even though results obtained 24 hours after harvest are a physiological "artifact", if they are reproduceable they would seem to have some value in this connection. Ezell and Gerhardt (39) did not recognize that it had already been reported (112) that English apples seem to have a climacteric rise while still attached to the tree. The possibility of an artifact for certain varieties of fruit still would remain, however.

Another significant value of knowing whether or not apples are pre- or post-climacteric is in connection with a possible relation to other physiological processes. For example, post-climacteric apples and pears do not respond to ethylene additions (71, 176). Another example is that the peak in the production of volatiles seems to follow the respiratory "hump" by several days at a high storage temperature (53, 179).

Effect of Temperature. In general, the respiration rate of deciduous fruits increases with a rise in temperature. It has been found, however, that the respiration rate at extremely high temperatures (80° F.), or at least the softening rate, of pears is reduced (121, 181).

Most chemical reactions, according to Vant Hoff's law, increase two or three times for every 10° C. rise in temperature. The exact increase for such a temperature rise has been called the Q_{10}. The Q_{10} for respiration seems to vary with species, variety, temperature of the storage and the nature of the atmosphere. It has been pointed out in the preceding section that the age of the fruit may also affect the Q_{10}. That is, one lot of McIntosh apples at 50° F. at its respiratory peak may respire faster than a second lot at 60° which is pre-climacteric.

In one of the earliest studies on apple respiration, Gore (58) found that the Q_{10} of apples varied from 2.10 to 2.40, depending upon variety. Another early study (138) showed a range from

2.4 to 2.9 for different varieties of apples. Kidd and West (98, 106) found a Q_{10} of 2.32 to 3.27 for English varieties in the temperature range studied. A Q_{10} as high as 4.1 has been found for peaches, although the value was not that high in the higher temperature range (67).

With some plant products there is an increase in respiration after the products have been subjected to cold storage temperatures and then removed to high temperatures. Although this effect has been noted in at least one instance (21), the subsequent respiration of apples, in general, does not seem to be affected by low temperature storage (34, 75, 124, 174). An effect of 32° F. storage on the subsequent respiration rate of pears at high temperatures has been noted in at least one case (121).

Effect of Carbon Dioxide and Oxygen. No attempt will be made here to review all the literature on the effect of various constitutents of the atmosphere on the respiration of fruits. The discussion will be confined to those studies which seem to have direct practical application in storage practice.

Hill (81) reviewed all the early literature on the effect of certain gases on the respiration of fruits and showed the inhibition of respiration resulting from low oxygen atmospheres. Kidd and West (97, 102, 109), working in England, have done the most extensive work on this problem from the practical standpoint. Their work led to the introduction of the technique which they call "gas storage" of apples and pears. This technique involves low oxygen and higher than normal concentrations of carbon dioxide at low temperatures.

The work by Kidd and West (95, 97, 102, 107, 109) and that done later in this country in California (3, 4), New York (178, 185, 192), Iowa (151) and in Canada (42, 148, 147) can be briefly summarized as follows.

Accumulations of carbon dioxide resulting from respiration tend to slow down the respiration process in apples and pears. The reason why these accumulations retard respiration is not known. It would be logical to assume that it is merely the law of mass action operating, were it not for the fact that respiration is not a simple one step process. Thornton (186) has suggested carbon dioxide accumulation has this effect through an influence on pH of the plant tissue. It is not clear, though, whether the pH change merely accompanies the accumulation or directly causes a retardation of respiration.

The exact amount of carbon dioxide that can be used safely over a long storage period depends upon species, variety, temperature and possibly the section in which the fruit is grown. Pears seem to be more tolerant of carbon dioxide at low temperatures than apples (107). The effect of 5% carbon dioxide on the storage life of pears is more striking than it is on that of apples because a temperature of 34° F. can be used with the former and temperatures as high as 40° F. must be used with the latter with that concentration of carbon dioxide. Some varieties of apple, *e.g.*, Baldwin and Rhode Island Greening, when grown in New York develop browning of the flesh when as little as 5% carbon dioxide is used at 40° F. Other varieties can withstand 10% carbon dioxide at that temperature. A carbon dioxide toxicity has been noted in English and Australian varieties of apples also (14, 23). In general, toxicity is greater at low storage temperatures. For example, New York McIntosh can withstand only 2% or 3% carbon dioxide at 32° F. but can tolerate up to 15% at 45° F. Carbon dioxide injury is evidenced by a browning of the flesh in some varieties and by rough depressed areas on the skin of other varieties.

Susceptibility to a given concentration of carbon dioxide may also depend upon growing or climatic conditions in the orchard. Iowa grown Jonathan seem to be able to tolerate 5% carbon dioxide at 40° F., but New York grown Jonathan develop a type of flesh browning when stored at that temperature in that amount of carbon dioxide (151, 178).

Sometimes deciduous fruits, *e.g.*, pears or sweet cherries, are exposed to high concentrations of carbon dioxide at approximately 50° F. for comparatively short periods of time. This technique is quite properly called "carbon dioxide storage" because this gas is artificially supplied from compressed gas in cylinders or from dry ice. With this treatment various fruits, *e.g.*, pears (52), plums (160), sweet cherries (56, 194), apricots and peaches (2, 57), are kept in excellent condition for short periods. Long treatments in storage with stone fruits are likely to result in abnormal ripening (57). Not only is there retardation in respiration rate but the fungus parasites are kept at a low level of metabolic activity and hence there is less decay in transit. As much as 40% or 50%

carbon dioxide may be used in these treatments. This is in contrast with long time storage where 5% or less of carbon dioxide is used with the various fruits.

A reduction in oxygen concentration around fruits in storage has a depressing effect on respiration independent of that of carbon dioxide (102, 107). In commercial practice a concentration of 2% oxygen is often recommended for apples and pears (107, 178). The apples through their own respiration process lower the normal amount of 21% to this figure.

If oxygen levels fall below 2% for more than a few days at a time with apples, a certain amount of anaerobic respiration is likely to ensue. This probably happens not because there is no oxygen available in the atmosphere but because there is a deficiency in the fruit's internal atmosphere. It has been shown that there is a lag of from 2% to 5% in oxygen concentration inside and outside apple fruits at low storage temperatures. At high storage temperatures there may be a differential of as much as 9% between internal and external atmospheres (104).

Stone fruits do not respond favorably to long time storage in low oxygen-high carbon dioxide atmospheres (6, 43), although one worker has claimed some slight benefit with peaches (79). When held in these controlled atmospheres for long periods these fruits may have a reduced respiration rate, but secondary effects make the fruits unpalatable. They do not seem to develop their proper eating quality, and often times there is a discoloration of the flesh around the pit. Little work, however, has been done on storage of these fruits in low oxygen atmospheres in complete absence of carbon dioxide. Successful storage of these fruits for long periods in the fresh state presents a real challenge to the physiologist.

Covering fruits like apples and pears with a wax emulsion may retard respiration through its effect on carbon dioxide and oxygen (104, 189). Attempts to lengthen the storage life of deciduous fruits from the standpoint of reduced respiration by waxing is sometimes dangerous. If the artificial wax coating is too thick, oxygen may become limiting, or toxic concentrations of carbon dioxide may accumulate (172, 189). It is true that proper application of such waxes may be of some advantage from the standpoint of lengthened storage life.

Effect of Ethylene and Acetylene. Ethylene has been used for many years to ripen certain fruits, *e.g.*, bananas and oranges (77, 80). Its use has not ever become widespread with deciduous fruits because the problem is usually one of delaying ripening instead of hastening it. However, it is sometimes used in ripening pears (1). Ethylene effects became of considerable interest when it was found, as will be shown in a later section, that many fruits evolve it as they ripen.

Kidd and West (99) were probably the first workers to recognize the possibility of one lot of apples stimulating the respiration of a second lot of fruit. They found a sharp induction of the climacteric rise in pre-climacteric fruit when the latter were exposed to ethylene vapors or ripe apple vapors, even at low storage temperatures (99, 105, 110). The effect of ripe peaches on unripe peaches has been noted in South Africa, but only very high temperatures (90° F.) were used (91).

Later work has elaborated on the conditions under which stimulatory effects on respiration may be expected. The magnitude of the effect resulting from exposure of apples and pears to ripe fruit emanations has been known to depend upon *a*) the age of the fruit; only pre-climacteric fruits are affected by ripe fruit emanations containing ethylene (71, 176); *b*) the variety; some apple varieties show more marked respiratory response than others (176); *c*) the temperature of storage; significant effects were observed at temperatures as low as 33° F., but more striking effects were observed at higher temperatures (175, 176); *d*) the number of ripe fruits; it required as few as 1% of the total fruits in the storage chamber to stimulate a respiratory rise; *e*) the variety of the ripe fruits; some varieties seem to evolve more ethylene than others (176); *f*) ripeness of the fruit supplying the ethylene (176). In view of the foregoing conditions, the effect of one lot of apples on another may vary from nil to a very significant effect.

The possibility of removing these ripe apple emanations from the storage atmosphere so that respiratory stimulation will not ensue will be discussed in a later section.

Artificial additions of acetylene seem to have the same effect on respiration of deciduous fruits as ethylene (28, 191). Danger of explosion would seem to discourage its use where respiratory stimulation is required, however.

Suggestions have been made on the nature of the relation of ethylene production to respiration, but no complete answers seem to have been offered (69, 122, 142).

Effect of Fruit Size. There seems to be little information on the effect of fruit size on respiration. It is quite often stated, however, that small fruits keep longer in storage than large fruits (59, 96). It should be recognized that factors other than respiration may be involved with the size factor. For example, small apples are usually firmer when placed in storage than large apples.

The author has conducted two experiments on the effect of fruit size with McIntosh apples and found a suggestion of more rapid respiration with large apples. The effect of fruit size should be studied further to discover the effect of exact leaf-fruit ratios on the tree on respiration in storage. From this preliminary work it would appear that respiration is not nearly so much a surface phenomenon as is transpiration (149).

Effect of Culture and Climate. Several studies (29, 94) have shown that the respiration rate of apples in storage was increased by application of nitrogen fertilizers to orchard trees. One study (59) indicated no response in respiration of fruit during the growing season, even though the nitrogen content of the fruit was markedly increased by nitrogen applications in the orchard. It would seem that, if conclusions were to be made on the effect of nitrogen fertilizers on keeping quality, the respiration measurements should be made during storage. Hulme (87) found an increased respiration rate of apples in storage after injecting individual limbs with urea. The author has sprayed individual limbs of Rhode Island Greening trees during the summer with urea solution and found an effect on respiration. He has also found a number of instances of increased respiration after harvest in McIntosh after applications of nitrogen fertilizer had been made in the orchard.

In one study in which it was not clear whether a definite potassium deficiency existed in the trees, an application of potassium fertilizers had no effect on subsequent respiration of apples in storage (29). Wallace (195) reviewed a considerable amount of literature and concluded that there was no clear-cut relation between mineral constitutents of apples and their potential storage life.

The exact effect of climate during the growing season on the respiration rate of apples or pears in storage has not been fully explored. Studies by the author indicate marked fluctuations in rate of McIntosh respiration picked from the same trees from year to year. One worker (197) has claimed that low respiration rate in storage was correlated with warm dry weather during the few weeks preceding harvest. One investigation with pears (161) showed a higher respiratory activity with pears on dry plots than fruits picked from irrigated plots, but there was no reduction in storage life in fruits from the dry plots. Effects of climate on keeping quality of fruits can not be correlated solely with respiration, since climate may also affect firmness of the fruit, susceptibility to storage disorders and possibly other fruit characteristics.

Effect of Injury. Many plant tissues respire faster when wounded. Most studies have shown little effect of bruising of apples on respiration rate (31, 92), but skin punctures or cutting the fruit does seem to stimulate respiratory activity (92). The author has examined the effect of bruising on McIntosh apples and has found no significant effect, whether or not they were bruised in the pre-climacteric or post-climacteric condition.

Severe injury to the cells of fruits, as may be caused by freezing followed by thawing, is very likely to induce a rise in the respiration rate (25).

Effect of Relative Humidity. It has been found (149) that the relative humidity of the atmosphere did not affect the respiration of apples unless the humidity became very low. This reduction in respiration could be attributed to physical changes in the character of the skin of the fruit which accompanied shrivelling. One worker (120) has reported increased respiration rate of pears when low humidities were used. Later work has indicated that the effects he noted were probably due to the very high temperature employed rather than the low humidity (181). Differences in relative humidities in the higher ranges (70%–100%) seem to be ineffectual in affecting respiration rate because the humidity of the intercellular spaces of the fruits probably stays close to saturation until the apples become somewhat desiccated.

Heat of Respiration. Some of the studies made on heat of respiration values were done by calculating the heat energy evolved by complete oxidation of a hexose sugar (67). Other studies have used calorimeters and measured the heat evolved (60, 61, 83, 163). The most exhaustive work on this subject has been performed by Green, Hulkill and Rose (60). They found that a ton of Stayman Winesap apples at 45° F. would evolve about 1550 BTU per 24-hour period. They also calculated the theoretical heat loss on the basis of oxidation of a hexose sugar and found fairly good agreement (less than 10% error). That is, it would seem that most of the energy lost in respiration is lost as heat.

A complete table of heat of respiration values for various fruits is available (158). Studies on heat of respiration should not overlook the fact that the heat evolved may vary with age of the fruit as well as the variety. Not even the variety is always specified (158). It should also be recognized that the Q_{10} is not a constant for even a given variety. Heat of respiration studies are still in progress, and eventually they will probably include the effects of these other variables.

A knowledge of the magnitude of heat of respiration is of utmost value to the refrigeration engineer who must compute refrigeration requirements. The author has used the figures of Griffiths and Awberry (61) and calculated the rise in temperature of apples in storage. Assuming that there is no heat loss (as there inevitably would be), a bushel of apples at 32° F. would raise their own temperature one degree F., in four days. At 66° F. the fruits would raise their own temperature two degrees in 23 hours. With more rapidly respiring fruits, like peaches, these temperature rises would be even larger. Calculations of this type are open to the criticisms noted above, but they illustrate the magnitude of the heat production that the engineer must deal with.

Relation of Respiration Rate to Length of Storage Life. Often there is a good correlation between respiration rate of apples and pears and softening and general storage life (26, 94), but frequently there doesn't seem to be a good correlation because of other factors limiting the length of storage life (161). Kidd and West (96) have gone so far as to forecast the potential storage life of an apple from a knowledge of the height of the climacteric rise, date of the rise, and the general level of respiration. The author (174) has found that the marketable life of McIntosh, as grown in New York, was at an end when this variety had evolved between 17.5 and 19.5 grams of carbon dioxide per kilogram of fruit when harvested each year at the same point on the respiratory curve. This represents an error of about 10% when complicating factors like low temperature breakdown were excluded.

It may be concluded that the respiration rate of deciduous fruits is one of the best single indices of metabolic activity that we have.

It should be recognized, however, that other factors such as transpiration, low temperature disorders and fungal invasion may be the determinants of length of storage life. A given lot of fruit may actually respire faster than a second lot, but because it is firmer at the start of the experiment it may have a longer storage life (161).

Practical Implications of Respiration Studies. Deciduous fruits do not respire at a constant rate at any one temperature. Whether this variation in rate can be used to practical advantage in determining when to pick certain varieties for storage remains to be seen. It would seem to be of doubtful value with some varieties but may prove to be of use with others.

The effect of a lowering of temperature on the respiration rate of fruits is the fundamental principle involved in cold storage. Many apple varieties will keep twice as long at 32° F. as they will at 50° F.

Retardation in the respiration rate of apples and pears by the use of relatively high concentrations of carbon dioxide and low concentrations of oxygen at a comparatively low storage temperature is the basis of the technique called "controlled atmosphere storage". This procedure has real commercial possibilities with certain species and varieties but not with others. One of the values of this newer storage technique is that there is a residual effect of storage. That is, apples removed from controlled atmosphere storage respire slower on the market place or in the hands of the consumer than do similar apples removed from cold storage (174). It is as though the fruits had been anesthetized during storage and never wholly recovered from the anesthetic.

When relatively high concentrations of carbon dioxide are used around fruits like pears and sweet cherries for comparatively short periods the treatment is called "carbon dioxide storage". Most sweet cherries now shipped from the West Coast are sent with this treatment in transit. Carbon dioxide storage differs from controlled atmosphere storage in that the carbon dioxide is added artificially, there is no control of oxygen, and the period of treatment is relatively short. Carbon dioxide storage would hardly seem to be an appropriate term for long time storage for a variety of apples like Rhode Island Greening where no carbon dioxide is used but the oxygen level is kept very low.

Evolution of ethylene by ripe fruits may significantly shorten the storage life of less ripe fruits stored with them. Where conditions are favorable for such an effect, as much as 25% of the storage life of a given lot of apples may be lost because of the presence of the ripe fruits.

The effect of culture and climate on respiration rate has not been fully explored. Under some conditions there may be measurable effects of variations in culture and climate on keeping quality.

The heat evolved in respiration is a very important factor for the refrigeration engineer. Heat of respiration must be compensated for in both precooling and storage of various fruits. Variations in heat produced within a species of fruit should be studied further with regard to variety differences and age of fruit.

PRODUCTION OF ORGANIC VOLATILE MATERIALS

Ethylene. Elmer (35) was probably the first to report that apples as they ripen emanate a gas that has effects similar to that of ethylene. Later work confirmed this report (72, 99, 105, 175, 176, 179). It has been shown that a sufficient amount of this gas is included in organic apple emanations to stimulate the respiration rate of other apples (99, 105, 175, 176, 179). The particular gas in these emanations responsible for these effects has now been definitely identified as ethylene (46, 47, 49, 72). Ethylene seems to be evolved not only by apples but by pears (50, 70, 72), peaches and plums (89, 90, 91) as they ripen.

Quantitative studies of ethylene production by apples and pears as they ripen (69, 140, 141, 142) have been made. In apples there is a progressive rise in the quantity produced to a peak followed by a decline in production. In McIntosh apples the peak seems to follow the respiratory peak by five days at a temperature of 20° C. (142). In pears the production curve seems to follow the respiratory curve very closely, the peak in production of carbon dioxide and of ethylene occurring at about the same time (69). Varieties of apples (141) and pears (69) having a long storage life seemed to produce less ethylene than those with a short storage life. One apple produced approximately one cubic centimeter of ethylene during its life after harvest (46). With pears it was found that the maximum amount of ethylene was produced at 20° C. with none being produced at 40° C. (69). Low oxygen atmospheres seem to markedly inhibit production of ethylene by pears (69).

Since complete absence of ripe fruit in the storage room is not always practical, the question arises as to the possibility of removing these organic apple emanations containing ethylene. Ventilation is not practical because of the extreme cost of refrigerating vast quantities of fresh air during the critical fall months of storage. Probably the first one to attempt air conditioning from this standpoint was Fontanel (44). He claimed success in removing the gas responsible for stimulating ripening of "fruit" by air conditioning with activated carbon. Repetition of this work with both lignite and cocoanut shell carbon did not show any promise in the high humidities involved in apple storage (176, 179). The work of Southwick (179) suggested the possibility of adsorbing bromine on the surface of activated cocoanut carbon, and air conditioning to remove ethylene. Air conditioning with this technique on small scale lots of apples works perfectly; only time will tell if this procedure has commercial possibilities (176). In testing the effectiveness of an ethylene absorbent or adsorbent the epinasty test (30) is very useful. Tomato plants are extremely sensitive to small concentrations of ethylene. Where air conditioning is not completely effective in removing the ethylene evolved by ripe fruit, tomato plants will show epinasty within a few hours at relatively high temperatures. This test has been used to considerable advantage by the author.

There is some evidence to the effect that ethylene may accumulate in toxic amounts in apple storage. The result is spotted fruit (113, 175).

Other Volatile Materials. While ethylene is one of the principal constituents of the emanations of deciduous fruits, there are other organic volatiles (196).

The odorous constituents of apples have been identified as consisting of the amyl esters of formic, acetic, caproic and caprylic acids as well as geraniol (154, 156). In peaches the odorous materials have been identified as linalyl esters of formic, acetic, valeric and caprylic acids. A high molecular weight aldehyde and an essential oil have also been isolated from the gases responsible for the odor of peaches (155). As yet, no studies have been made on the progressive changes of the various fractions of these odorous gases as the fruits ripen. The odorous substances are presumably produced in maximum amounts when the fruits are eating ripe.

Acetaldehyde has been identified as a constituent of apple and pear flesh (40, 51, 54, 76, 85, 135, 154). There seems to be more of this aldehyde in pear flesh than in apple (51). The exact role of acetaldehyde in fruit metabolism is not yet fully understood, although the suggestion has been made that it is present as a normal intermediate product of respiration (41). Most investigators of this topic seem agreed that there are traces of this aldehyde in normal apple and pear flesh and that during senescence (143) or in low oxygen atmospheres (182) or in fruits affected with apple or pear scald (51, 76, 183) there may be accumulations. The suggestion that a disorder like pear scald may be caused by accumulations of acetaldehyde (76) has been challenged by at least one investigator, Trout (188), who has pointed out that these accumulations may not necessarily be the cause of scald but may merely accompany the disorder.

Traces of ethyl alcohol have been found in normal pear and apple flesh (40, 135, 182), but it is not likely that appreciable quantities accumulate except under very low oxygen conditions (182) or during senescence (143). Vapors of ethyl alcohol in fruit tissue seem to retard respiration (103). It is very likely that the off flavors encountered in fruit that has been stored in anaerobic conditions or that has developed flesh breakdown from one cause or another is due to ethyl alcohol or acetaldehyde, or both (51).

The only specific gases emanating from fruits that have been measured quantitatively are ethylene and acetaldehyde. Measurements have been made of total volatiles and we know little of the quantitative or qualitative production of the various volatiles other than ethylene and acetaldehyde (45). Measurement of these total volatile materials is usually made by adsorption in sulphuric acid followed by oxidation of ceric sulfate (51, 179). Ethylene is not included in these measurements unless "activated" sulphuric acid (silver and nickel sulfates added) is used (51). Sometimes total volatiles are determined by complete combustion of the gases following removal of carbon dioxide evolved in respiration (48). While these measurements of total volatiles are valuable they would be more so if the quantities of constituent gases were known For example, the total quantity of volatiles might be quite low, yet a specific volatile responsible for some disorder (such as apple scald) might be present in considerable quantities from a relative standpoint.

Measurement of total volatile materials emanating from apples has shown that temperature has a very striking effect. With Golden Delicious apples almost twice as much was produced at 36° F. as at 31° F. (53). With McIntosh apples twice as much was produced at 40° F. as at 32° F. (179). It has been noted that there was a residual effect of storage treatment on the subsequent evolution of total volatiles. It was found that McIntosh apples stored at 32° F. evolved less total volatiles when removed to a high temperature than did similar apples which had been held in 40° F. storage (179). This finding may offer a clue as to why McIntosh apples held in cold storage never seem to develop their full aroma. The same thing has been observed with McIntosh apples held in controlled atmosphere storage as compared with storage in air (179). While it is true that controlled atmosphere apples may not be as aromatic as apples of the same degree of ripeness held in ordinary cold storage, they are longer lived upon removal from storage and evolve volatiles over a longer period of time. The fact remains, however, that they are not so aromatic as cold storage apples upon removal from storage.

Injured fruit seems to produce volatiles more rapidly than uninjured fruit (53). Whether this fact has any significance from the standpoint of loss of quality as a result of injury remains to be shown.

Practical Implications of Organic Emanation Studies. It has been pointed out previously that sufficient ethylene may be evolved from one lot of fruit to stimulate the ripening of other fruits. The practical value of ethylene removal from fruit storages has not yet been demonstrated.

The storage disease of apples called "scald" is caused by accumulations of certain gases around the fruits. These gases are evolved by the fruits themselves. The exact gas or gases responsible for this disease are not yet known. Their identification would help considerably in a better approach to control of the disorder. Attempts at control are now made by wrapping or mixing the fruit with paper impregnated with mineral oil. The possibility of air conditioning for removal of these noxious gases should be explored.

The organic emanations that contribute aroma are of considerable importance from the standpoint of fruit quality. The storage treatments that are most likely to give maximum production of these aromatic constituents as well as long keeping have not yet been very thoroughly investigated.

CHEMICAL CHANGES DURING STORAGE

Enzyme Action. Many of the chemical changes occurring in fruits during storage can be attributed to enzyme action. For example, the change from starch to sugar, sucrose to invert sugar, or of proto-pectin to pectinic acid is due to enzyme action. However, very little work has been done on enzymes as such or their specific responses to changing storage conditions. The experimental work has concerned itself with the net result of these enzyme reactions. For example, in studying the softening of fruits in storage, the investigational work has concerned itself with increases in pectinic acid rather than in studying the specific activity of the protopectinase enzyme. The results of some of these enzyme actions are discussed in the sections to follow.

Some work has been done on oxidase and catalase activity in apples and pears in relation to their keeping quality in storage. With pears it was found that the curve for catalase activity was U-shaped. The minimum rate of catalase activity was found to occur at or near the time the Bartlett pear should be harvested for storage. There was a subsequent rise in catalase activity after this minimum point was reached (37, 38). There was a more or less continuous declining rate in oxidase activity in pear fruits throughout the whole growing season. Oxidase activity in pears continued to decline in rate during storage (37, 38). In apples it has been found that there is a continuously rising catalase activity during the growing season and after harvest. Oxidase activity was found to decrease during the growing season and might or might not increase when the fruit becomes more mature. Fruit harvested when fully mature usually showed a higher catalase activity and a lower oxidase activity in storage than fruit picked when less mature (39). The full significance of oxidase and catalase activity has not been established for fruits in storage. It seems that in apples and pears oxidase and catalase activity have no apparent direct relation to respiration or to each other (39).

Sugars. The major portion of the dry matter of deciduous fruits is made up of carbohydrate material. Of this carbohydrate material sugars compose the largest fraction. The sugars commonly found in these fruits are glucose, fructose and sucrose (184). The exact proportion of each depends upon species and to some extent upon variety and maturity (184). For example, fructose and glucose are the principal sugars in apples and pears whereas sucrose is the principal one in peaches (132, 184). The amounts and proportions of these sugars also vary in different parts of the fruit (73). For example, in pears fructose is the main sugar in the flesh whereas dextrose is the main sugar in the skin (132). The blushed side of fruits is usually higher in total sugars than the unblushed (shaded) side (12). Fruits vary in their concentrations of total sugars from year to year and from orchard to orchard (10).

The usual procedure in apples is for the total sugars to increase for a time during storage and then to begin to decrease (10, 78, 82, 126). Increase in total sugars during the early stages of storage is due to hydrolysis of starch (11) in apple, and in pear it is probably due to a breakdown of sorbitol (115, 133). The normal sugar changes can in a general way be retarded by lowering the storage temperature (126) or by modifying the atmosphere (136, 137). Ethylene treatment of preclimacteric fruit tends to cause an accelerated loss of sugar through respiration (32). It is very difficult, however, to always correlate storage treatments with sugar changes in so far as total amounts are concerned (126). During storage there is apparently considerable conversion from glucose to fructose or possibly *vice versa,* particularly during senescence (11, 145).

There have been some attempts to correlate carbohydrate composition with potential storage life but they have not always been convincing. For example, it has been claimed that when the nitrogen content of the apple fruit was high, sugar and acid losses were more rapid in storage (10). It was also predicted that when sucrose content is high in apples and nitrogen low, storage life will be longer (78). It may some day be definitely established that carbohydrate composition has a definite relation to potential storage life, but it will probably still be recognized that other factors may be the determinants.

One of the causes of death of fruits is often presumed to be lack of respirable material. For example, it has been stated that exhaustion of the sucrose content of apples coincides with death of the fruit (78). However, there are reports that fructose is the respirable material in apples and that there is a conversion of glucose to fructose even during senescence (144, 145). Another debatable point that arises here is—just when is an apple "dead"? The author has noted that there may be as much as 8% total sugars left in apples when they are in a degenerated condition long past the state of marketability.

Starch. Many varieties of apples and pears have starch in the tissues of the flesh (1). It has been reported that some varieties have as much as 1% when placed in storage (11). During storage this is converted into sugars, as noted above. The presence of starch in stone fruits during storage has not been reported.

Acids. During storage of deciduous fruits there is a gradual loss in total acidity (33, 78, 93, 126) and a rather small increase in pH (22, 93). The acid involved is primarily malic, although there have been some reports of traces of tartaric, citric and succinic acids in deciduous fruits (16, 139). Bigelow and Dunbar (16) offer a full bibliography on the work on fruit acids up to 1917 and offer data on the percentages of acids in the common fruits. Acids decrease during storage presumably because they are a partial substrate for respiration (126).

Various workers have tried to associate acid content or acid changes with the storage life of fruit. One report (33) claims that the ratio of total acids to dissociated acids determines the keeping quality of fruits in storage. Another (62) claims that internal breakdown in apples is associated with high acidity. This worker (62) recommends that in view of this fact apples should have a period at high temperatures to reduce the acid content of the fruit before low temperature storage. This recommendation based on theory is not in accord with the observed facts in connection with general longevity of fruits. It is quite possible, however, that fruit acidity may have some bearing on specific storage disorders. It has been shown, for example, that the amount of the storage disorder called Jonathan spot was directly correlated with the amount of acid (152). There was more Jonathan spot on samples entering storage with a low acidity and on samples which lost the smaller amounts of total acid during storage (152). The authors also found a small amount of soggy breakdown when the total acid loss was small in storage. How much these correlations are pure coincidence and how much a cause-effect relationship is not clear as yet.

Attempts to correlate storage treatments with acidity changes have not given very promising results in experimental storage work (93, 126), although, in general, storage in high temperatures results in more rapid acidity losses (152).

Pectin. As deciduous fruits ripen in storage there is a change from proto-pectin to pectin. This pectin change is usually offered as one of the primary reasons for softening of fruits (7, 24). As the proto-pectin is dissolved away from the middle lamella, the cells of the flesh become less firmly cemented together and softening ensues. Various workers (7, 24, 121, 153, 177) have found a good correlation between fruit softening and increase in pectin. When fruits become over-ripe, pectin reaches its highest percentage and then declines in amount. There is a very rapid disappearance of this soluble pectin or pectinic acid in over-ripe apples (192). Whether it breaks down into its component parts is not known, but it presumably does. If it does, it may partially account for the fact that the sugar content of over-ripe fruits is often unexpectedly high.

Soluble pectin increases can usually be rather closely correlated with storage treatments. For example, the increase is very much diminished by low temperature storage (121) or by controlled atmosphere storage (36, 177, 193). While there is normally a more rapid increase in pectin at higher temperatures, if the temperature goes too high (80°) there is a retardation in activity of the proto-pectinase enzyme that produces water-soluble pectin (121). The softening rate decreases also at these very high temperatures with pears (121, 181). Sometimes the storage treatment has a residual effect on subsequent pectin changes in certain fruits. In apples it has been observed that controlled atmosphere treated fruits do not soften as fast as would be expected upon removal to air at high temperatures and that protopectin hydrolysis is slower than would be expected (193). In plums the normal pectin increase at high temperatures seemed depressed after storage at low temperatures in one study (68).

In stone fruits the softening rate is very rapid and the pectin or pectinic acid increase is normally so, too (7, 177), but attempts to correlate differences in rate of softening between varieties on a basis of this factor have not been altogether successful (64). Obviously there must be other factors involved in softening besides merely the pectin transformations. Differences in morphology and anatomy possibly would help to explain varietal differences in softening rate.

Waxes. On the outer surface of the epidermis of the apple fruit there is a layer called the cuticle. Considerable soft wax may develop on the surface of this cuticle. This waxy coating has been found to consist of ursolic acid, "oily fraction" and "total ether soluble extract". An ether extract does not contain a ketone in appreciable amounts, but the bulk of the extract in apples and pears contains n-nonacosane (127, 128). During storage of apples all these constituents increase in amount, but the oily fraction increases at a faster rate than the ursolic acid (129). This shift in amount and proportion of oily fraction probably accounts for the increasingly greasy feeling and appearance of some varieties of apples in storage. The total amount of cutin also increases during storage of apples (130). Different varieties of apples vary widely as to their amounts of ursolic acid, oily fraction and total ether extract (129), and the amounts vary within varieties from season to season and from orchard to orchard (129, 130).

Several workers (127, 146) have attributed variations in physiological processes to variations in the nature of the waxy coating of fruit, but the full relation of this factor to the physiology of the fruit has not been very fully explored as yet.

Nitrogen. Changes in the total nitrogen in the flesh of apples does not change very much during storage, but there is a marked change in the protein fraction (8, 9, 86). The insoluble (protein) nitrogen increases in amount. This increase in apples and pears follows the respiratory rise very closely (114). The increase in protein nitrogen is due to a decrease in soluble nitrogen (86). The rise in amount of protein nitrogen can be induced in preclimacteric fruits by addition of ethylene, or it can be delayed by use of controlled atmosphere storage (114). While storage treatments may be correlated rather well with protein nitrogen changes, it does not seem likely that determinations of this change would be as valuable as respiration measurements, because the magnitude of protein differences is not so marked as respiration differences (88).

Attempts to correlate the content of total nitrogen with keeping quality of fruits in storage have given conflicting results. Several English workers (9, 86, 87) have associated poor keeping quality with high nitrogen. The work of Gourley and Hopkins (59) typifies most of the work in this country which shows no relation between fruit nitrogen and keeping quality. Investigations on the McIntosh variety of apples by D. Boynton and the author (unpublished) show that differential nitrogen treatments in the orchard may show no effect on keeping quality because the amounts of nitrogen in the tree may not be affected. When different levels of leaf nitrogen (sampled in July) were attained, there was a strong suggestion of reduced fruit firmness and ground color (yellow) development with the higher nitrogen applications in the orchard. When leaf nitrogen was high there was a tendency for greater susceptibility to brown core (a low temperature disorder) in this variety. This variety is very sensitive in other ways to nitrogen level differences, so it does not necessarily follow that these findings will apply to other varieties.

Ash. There are not marked variations in the ash content on a dry weight basis of fruits as they ripen in storage (19). There have been some attempts to correlate storage life with amounts of mineral constituents in the fruit. For example, one report (19) claimed that long keeping of apples was associated with high potassium and phosphorus in the fruit. Other reports (29, 84) have shown no correlation between mineral content of apples and storage life. It is true that a real deficiency of a mineral like potassium (195) might affect the storage life of fruits, yet Wallace (195) points out that high potassium fruits may keep better than low potassium fruits at low temperatures and poorer at higher storage temperatures. He concluded that there was no clear cut relation between mineral composition and length of storage life.

Practical Implications of Studies on Chemical Changes. Studies on chemical changes occurring in storage have helped us understand the effect of storage treatments on softening. Methods of retarding the proto-pectin to pectin change is sought by all storage investigators. In general, attempts to correlate other chemical changes with specific storage treatments have not been very fruitful from a practical standpoint. Effects of storage treatment on chemical changes that affect quality have not been explored so fully as they might have been. While some work has been done on the effect of storage treatment on vitamin changes, comparatively little has been performed as yet on the general nutritional value of various fruits when stored in different ways.

SUMMARY

The transpiration rate of deciduous fruits determines how soon a given lot of fruits will become unmarketable because of shrivelling or wilting. This loss of water results not only in a loss of eating quality but also in unsightly appearance of the fruit. The transpiration rate is influenced by the vapor pressure deficit, air movement and certain internal fruit factors. Transpiration may be held at a very desirable minimum by storage in relative humidities approaching saturation, but surface growing fungi are likely to be a source of trouble. Work on methods of control of these surface molds by plant pathologists is needed.

The respiration rate of deciduous fruits is probably the best single index of metabolic activity in relation to potential storage life. It should be recognized that respiration rate is not the sole determinant of length of keeping of these fruits, however. The respiration rate of a given lot of fruit in storage depends upon its age, climate during the growing season, temperature, carbon dioxide and oxygen level, and ethylene concentration. It is not entirely clear what the effects of bruising, fruit size and nitrogen level in the tree are on subsequent respiration of fruits in storage. While considerable work has been done by physiologists on the nature of plant respiration, still more work is needed. When the complexities of fruit respiration are fully understood it is very possible that the pomologist can suggest better ways of handling fruits in storage. For example, one of the greatest challenges offered to the physiologist in this connection is that of finding a way to reduce the metabolic activity of stone fruits without incurring unfavorable secondary reactions. This would permit long time storage in the fresh state.

Production of organic volatile emanations by deciduous fruits is of considerable interest to the pomologist. Under some circumstances, one lot of apples or pears may evolve enough ethylene to stimulate the ripening of a second lot in storage. The odorous emanations of various fruits are of considerable importance from the standpoint of eating quality. Not very much is known about ways in which maximum aroma may be produced without unduly sacrificing length of keeping in storage. Accumulations of certain volatile materials, as yet unidentified, cause a storage disorder known as "scald" on many varieties of apples.

Deciduous fruits undergo many chemical changes as they ripen. Many of the analyses of the various constituents of these fruits have not been of great benefit in interpreting experimental results in storage. A notable exception is that of the proto-pectin to pectinic acid change which accounts at least in part for the softening of fruits in storage.

Improvements in storage techniques are the responsibility of the physiologist, the pathologist, and the refrigeration engineer. Not only must studies of normal metabolism be continued, but causes of pathogenic or physiogenic disorders must be constantly investigated. Tremendous economic loss is caused by these disorders in storage.

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ANTIBIOTIC SUBSTANCES PRODUCED BY SOIL BACTERIA

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INTRODUCTION

The name "antibiotic substances" refers to a group of substances of divergent chemical nature which are produced by certain species of micro-organisms and which have definite bactericidal or bacteriostatic properties against other groups of micro-organisms.

The enormous popularity that this group is enjoying at present is largely due to the fact that certain members of it, especially penicillin, not only are potent bactericidal agents but also possess remarkable therapeutic potentialities. The feverish research being conducted in this newly discovered field is at present directed mainly toward the discovery of new chemotherapeutic agents, and in many instances is approaching the dimensions of a scientific gold-rush.

Although the possibilities for discovery of such antibiotic substances are limitless, it is by no means certain that this field will prove to be the rich mine for therapeutic agents that many investigators believe it to be. Currently there are no scientific facts to justify such expectations. The mere fact that bactericidal substances are excreted by living organisms unfortunately does not necessitate that such agents have therapeutic value or would be less toxic for humans than bactericidal chemicals, such as phenols, mercurials or certain detergents. On the other hand, the mere fact that the organism which excretes such substances apparently is not affected by them, indicates that there is a marked specificity for destruction of certain living cells, whereas other living cells are not affected. Such a high degree of specificity is rarely encountered among the common bactericidal chemicals and, as is the case with penicillin, opens the possibility that certain antibiotic agents may exhibit specificity for certain disease-producing bacteria and be relatively non-toxic to human cells.

Although the existence of antibiotic substances has been known since the early days of bacteriology, it was not until recently that the field was explored. Undoubtedly the stimulus for this new exploration was the sudden revival of interest in chemotherapy, growing from the discovery of sulfa drugs. Sulfanilamide was known to the chemists for a long time, but it was not until about thirty years after its discovery that its therapeutic potentialities were recognized. This discovery at once revived the almost dormant science of chemotherapy and gave the research worker in this field renewed confidence in the belief that infectious diseases could be cured by the use of chemicals.

The bacteriologist, by the nature of his profession, was among the first to realize the major importance of the discovery of sulfa drugs, and the short step from the field of sulfa drugs to that of antibiotics was a logical development.

In the early days of bacteriology, more than sixty years ago, it was observed repeatedly that when several bacterial species were allowed to grow together on the same nutrient plate, a clear and sterile halo sometimes developed around the colonies of certain species. Apparently the bacteria of such a colony excreted a substance into the medium which prevented bacteria of non-related species from growing close to such a colony. This phenomenon, referred to in the older literature as "bacterial antagonism", was studied quite extensively at the turn of the century. A large number of such "antagonistic" strains was isolated. Each of them specifically inhibited the growth of one or more species under certain conditions. A considerable strain-specificity was observed among these antagonists, and usually only a few strains of the same species possessed such antagonistic properties.[1]

Already early investigations revealed that the phenomenon of bacterial antagonism, in general, could not be explained by the assumption that the medium was exhausted by the growth of the antagonist, nor could it be explained by the excretion of the usual metabolic products of the antagonists, such as organic acids, alcohol, ammonia or indole. Soon it was realized that certain strains under specific conditions of growth are able to excrete into the medium, substances of high bactericidal potency for other species. Garré (2) pointed out as early as 1887 that the presence in the soil of such

[1] For an excellent review on bacterial antagonism see: 145.

bactericidal substances, excreted by certain types of soil bacteria, may be the explanation for the rapid destruction of pathogenic organisms when they are added to the soil. The same investigator expressed the opinion that eventually such bactericidal agents, excreted by soil bacteria (antibiotics), might be used as valuable therapeutic agents for treatment of bacterial diseases.

Although considerable work was done in this direction, little progress was made. Attempts to isolate and purify the bactericidal products usually met with little success, and finally the idea was entirely dropped as being impractical. Pyocyanase, a thermostable agent of lipoid nature, excreted by certain strains of *Pseudomonas aeruginosa,* was probably the first antibiotic substance isolated at that time with any degree of purity. Not until five years ago, however, was the study of bacterial antagonism renewed, and special emphasis was then laid on the isolation and purification of antibiotic substances from culture media.

TYROTHRICIN (GRAMICIDIN and TYROCIDINE)

Discovery

During the early thirties Dubos, at the Rockefeller Institute, was conducting studies on an enzyme which digested the capsular polysaccharide of *Pneumococcus*—Type 3. This enzyme was excreted by a spore-forming bacillus, isolated from soil. Its action was not restricted to hydrolysis of the purified polysaccharide *in vitro;* the enzyme was also able to digest the capsule as soon as it was produced by the living and multiplying pneumococcus. Not entirely satisfied with these results, Dubos wanted to go one step further. He considered the possibility that there also might exist bacterial enzymes capable of attacking not only soluble and isolated compounds but even the intact living cells of unrelated microbial species. With this idea in mind Dubos took soil samples and added to them, during the course of a whole year, suspensions of living pneumococci in the hope that, if such a pneumococcus-digesting organism was present in the soil, it would accumulate (14). However, Dubos never found such an enzyme, but, instead, isolated an organism from this soil mixture, which later was called *"Bacillus brevis"* (14, 15, 16, 17). Undoubtedly this organism had been present in that soil mixture in large amounts before the feeding experiment was started. In all probability it did not "accumulate". *Bacillus brevis* does not feed on pneumococci, nor do pneumococci in any way incite excretion of a bactericidal substance by it; neither does it excrete an enzyme that digests living bacteria. Unintentionally Dubos had isolated a pure representative of the group of "bacterial antagonists", known since the time of Pasteur, and a study was undertaken in order to determine the properties of the bactericidal agents excreted by this antagonistic strain.

It was found that filtrates of a culture of *Bac. brevis* contain a substance which is highly bactericidal for a large group of Gram-positive bacteria, including pneumococci, streptococci and staphylococci. After much painstaking work, Dubos and his co-workers finally succeeded in isolating a crude material from cultures of it, which contained all the activity present in the original broth. This crude water-insoluble material, highly bacteriostatic for a large number of Gram-positive bacteria, was given the name "tyrothricin". Later it was separated into two crystalline fractions, gramicidin and tyrocidine (27, 18).

The same organism, as well as the two active principles, were isolated by the present author, independent of Dubos' work and at about the same time, during a study on inhibition of encapsulation of Friedlaender's bacterium by excretion products of soil bacteria (25, 26). It was observed that Friedlaender's bacterium, which under normal conditions produces enormous capsules of polysaccharides, failed to do this when grown in symbiosis with a soil bacillus which had been previously isolated from soil. Although no capsules were produced, the growth of Friedlaender's bacterium was not impaired by the presence of this soil bacillus. After an extensive study the active agent, excreted by this soil bacillus and responsible for this inhibition of encapsulation, was isolated, purified and crystallized. It was found to be identical with Dubos' tyrothricin (32).

Description of Bacillus brevis and the Conditions of Tyrothricin Formation

Colonies growing on nutrient agar: colonies circular, 1–2 mm. diameter after 24 hours incubation at 37° C.; flat, smooth, entire edges, white, pearl-like glistening, sometimes becoming slightly yellowish-brown on further incubation; mesophylic, strictly aerobic.

Morphology: actively motile, Gram-negative rods, single or in pairs; central spores; rods distinctly swollen during sporulation; endospores are ellipsoid.

Growth in nutrient broth: moderate clouding, slight pellicle as well as sediment.

Carbohydrates: no fermentation or acid production.

Litmus milk: after two days: reduction of litmus, milk clots, increasing peptonization, alkalinization.

Nitrate reduction: active reduction and nitrite formation.

Gelatin liquefaction: complete liquefaction of peptone-gelatin with considerable formation of ammonia.

Starch: no appreciable hydrolysis.

Indole: no formation in tryptophane broth.

Catalase: positive.

The foregoing description of the organism producing tyrothricin (14) is not in every respect identical with that of *Bac. brevis* in Bergey's "Manual of Descriptive Bacteriology", although the organism undoubtedly belongs in the large "brevis group". In the absence of criticism of taxonomists the organism has been generally accepted as *Bac. brevis.*

Relatively little is known of its physiology. A thorough systematic study on the conditions under which gramicidin and/or tyrocidine are produced has never been made. In shallow stationary cultures, one to two inches deep, tyrothricin is produced on a wide variety of media, such as prepared from peptones, tryptone, yeast extract, hydrolysed casein or gelatin, whey, potato extract or vegetable juices. Also, good yields are obtained with pure synthetic media containing glucose (1%) as the source of carbon, and one amino acid, such as asparagin ($\frac{1}{4}$%), glycine or glutamic acid ($\frac{1}{2}$%) as the source of nitrogen, together with small amounts of inorganic salts, such as those of K, Mg, Na, Mn, Fe, Ca, $-PO_4$, $-SO_4$ or $-Cl$ (44).

Tyrothricin is elaborated already during the early logarithmic growth-phase and consequently cannot very well be a degradation product formed during autolysis. It accumulates during active growth of the organism and does not seem to be destroyed by further incubation. After one to two weeks there is usually no further noticeable increase in quantity. Yields of 300 mg. to 1 g. per liter of culture medium have been reported.

Growth of the bacterium involved and tyrothricin production do not always go together. Stokes and Woodward (44) showed that luxuriant growth without tyrothricin production occurs in aerated, submerged cultures of *Bac. brevis* on a large variety of complex nitrogenous media, in small tanks provided with stirring and aeration devices. However, when synthetic media of the composition given above were substituted, yields of 100 to 300 mg. per liter of culture medium were obtained by the submerged culture methods in tanks. No satisfactory explanation for this behavior can be given at present.

There are indications that tyrothricin produced by the submerged method contains a greater percentage of gramicidin than that produced by the stationary method. Tryptophane can not serve as a source of nitrogen in synthetic media for tyrothricin production. This is the more remarkable because this amino acid is one of the most important building components of gramicidin and tyrocidine.

In general it can be said that both gramicidin and tyrocidine are formed at the same time in a wide variety of media and under most divergent conditions. Tyrocidine usually is present in considerably larger amounts than gramicidin, about 4: 1. No thorough study has ever been made to determine under what conditions production of tyrocidine is favored and under what conditions production of gramicidin is stimulated. One reason for this may be the fact that at present no suitable method is available to determine the concentration of both antibiotic agents separately in crude filtrates containing both.

Although gramicidin and tyrocidine are the main antibiotic fractions in the filtrates of *Bac. brevis,* there are undoubtedly other antibiotic fractions which have not been sufficiently investigated. Dubos, for instance, found a water-soluble fraction, behaving like a protein, and Referent has repeatedly found small amounts of an active fraction which is soluble in hot alcohol, but precipitates upon cooling. Both antibiotic fractions need further study.

Isolation of Bacillus brevis from Soil

A suspension of one part of ordinary garden soil in five parts of water is heated for ten minutes in a water-bath at 80° C. in order to destroy all non-sporulating species of bacteria. This pasteurized soil suspension is then streaked over the surface of several peptone agar plates with a platinum wire. After 48 hours incubation at 37° C., the round flat and white colonies with entire edges and typical pearl-like glistening are isolated and inoculated into tubes with a 1% peptone–1% glucose–bromthymolblue–containing nutrient broth. When a number of different soil samples are used, little difficulty is encountered in finding separate colonies of the description given above. Tubes wherein no acid is produced and in which no pronounced pellicle growth is observed, usually are those of pure cultures of *Bac. brevis*. In order to prove this, one ml. of a three-day-old culture is added to 100 ml. of nutrient broth (without serum), inoculated with a Gram-positive organism such as *Pneumococcus* or *Streptococcus*. A dilution 1:100 of a three-day-old culture of *Bac. brevis* should exert enough bacteriostatic action to prevent growth of most of the Gram-positive species. Using this technique, Referent was able to isolate *Bac. brevis* from more than half of a large number of soil samples taken at random from several locations.

Preparation of Tyrothricin (Stationary Culture-Technique)

A very suitable and inexpensive medium for cultivation of *Bac. brevis* on laboratory scale is a 2% gelatin–½% casein solution (both commercial grades) in tap water. By addition of the required amount of trypsin, the proteins are digested by allowing the medium to stand for five hours at 37° C., adjusting the pH repeatedly to about 7.5 with NaOH. After digestion the medium is distributed in one-liter flasks or bottles in layers about two inches deep. Recently the use of press juice obtained from waste asparagus butts was advocated. This medium seems to give excellent yields of tyrothricin but is not readily available for laboratory-scale production at present. After sterilization each flask is inoculated with about 10 ml. of a 24-hour stock culture of *Bac. brevis* in the same medium. The flasks are incubated preferably at 35° C. for 7 to 14 days.

The content of the flasks is then harvested, the pH adjusted to 4.5 to 5 with concentrated HCl, and the heavy precipitate is allowed to settle overnight. The supernatant liquid is then syphoned off and discarded. To the remaining heavy suspension of tyrothricin and bacterial debris, methanol is added until a concentration of 60% is reached. This mixture is stirred frequently and allowed to extract for 24 hours, preferably at somewhat elevated temperature. The supernatant liquid, rich in tyrothricin, is then syphoned off and the remaining debris extracted once more with 70% methanol. The debris is separated from the second extract by centrifugation.

The combined alcoholic extracts are filtered through paper, and an equal volume of 1% NaCl solution added.[2] A precipitate of tyrothricin is formed which is allowed to settle overnight in the refrigerator. The clear supernatant liquid is decanted and the precipitate collected on a Buchner funnel, washed twice with distilled water, and dried. When thoroughly dry, the powder obtained is extracted twice with ether for removal of inactive substances of lipoid nature. Tyrothricin is soluble in 95% alcohol up to at least a 5% solution. A considerable part of a dark pigment may be removed from such a solution by treatment with norite. The usual yield is one gram of tyrothricin per liter of culture medium.

Separation of Purified Tyrothricin into Gramicidin and Tyrocidine

Gramicidin is soluble in hot absolute acetone; tyrocidine is insoluble in acetone, provided the last traces of water have been removed and the tyrothricin itself is thoroughly dried over P_2O_5 in vacuum.

By extraction of dry tyrothricin with warm absolute acetone, a separation of both components can be achieved. To the acetonic solution of gramicidin is now added one volume of dry ether, and the precipitate is removed. The remaining solution is then evaporated under vacuum until a small volume remains. Crystalline gramicidin is obtained upon cooling. Further purification may be obtained by recrystallization from warm absolute acetone.

The absolute acetone–insoluble tyrocidine fraction is dissolved in about four times its weight of boiling absolute alcohol. A crystalline precipitate is obtained by addition of an alcoholic solution of HCl. The crystals of the HCl salt of tyrocidine may be recrystallized from absolute methanol.

Determination of Gramicidin and Tyrocidine in Mixtures and in Crude Filtrates

Optical rotation method: An alcoholic solution of pure gramicidin has an optical rotation of +5, whereas tyrocidine under the same conditions gives an optical rotation of − 105. Tyrothricin or any other mixture of gramicidin and tyrocidine gives optical rotations between these extremes, and from the values observed the concentration of both components can be calculated. This method, of course, can be used only when no inert or other optically active impurities are present in the preparation.

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[2] Instead of precipitation with 1% NaCl solution, the alcohol may be evaporated until only a water phase remains.

Hemolysis method: The tyrocidine concentration found by the above method can be checked by another method based on the fact that tyrocidine is extremely hemolytic whereas gramicidin does not show hemolysis under the same conditions. An amount of 0.05 mg. tyrocidine added to 1 ml. of a suspension of fresh rabbit erythrocytes in 5% glucose solution causes complete hemolysis when incubated for two hours at 37° C. Even 0.005 mg. per liter causes partial hemolysis. Under the same conditions 0.5 mg. of gramicidin does not cause hemolysis.

This hemolytic property of tyrocidine has been utilized as a basis for a quantitative method of determining tyrothricin in culture filtrates of *Bac. brevis* (11). This, of course, is basically wrong, since, *a priori,* one may not assume that tyrocidine and gramicidin are excreted during the whole growth process in a constant ratio. Even different batches of tyrothricin, used for comparison, do not always contain the same ratio of tyrocidine and gramicidin. However, for the determination of tyrocidine, either in crude filtrates or in tyrothricin preparations, this method of Dimick would be well adapted when comparisons are made with pure tyrocidine instead of with tyrothricin. The method, in short, is based on measuring the degree of hemolysis of a standard suspension of rat erythrocytes under well defined conditions of medium, temperature and time of exposure. The degree of hemolysis, measured by photoelectric-colorimetric determination of light transmission, was found to be proportional to the amount of tyrothricin (tyrocidine?) added to the erythrocyte suspension. From the results obtained, a straight line calibration curve, covering the range of 4 to 10 micrograms per ml., was obtained. The amount of hemolysis produced by an unknown solution is measured and the tyrothricin (tyrocidine !) content is read directly from the calibration curve. For the assay of culture media, 1 ml. is added to 9 ml. of 95% alcohol. After thorough shaking the debris is removed by centrifugation and ½ ml. of the clear supernatant liquid is added to the standard erythrocyte suspension. For determination of the calibration curve the same broth and alcohol concentration is used. The accuracy is ± 5%. No satisfactory method for the determination of gramicidin, independent of tyrocidine, is known.

Chemistry of Gramicidin and Tyrocidine

Considerable work has been done to reveal the chemical structure of both gramicidin and tyrocidine. Although much progress has been made, insurmountable difficulties are still encountered, due to the nature of the compounds. Both gramicidin and tyrocidine are polypeptides of high molecular weight. The molecular weight of tyrocidine is 2,500 to 2,700; that of gramicidin is not definitely known but most likely is 1,500 or multiples of this value (51, 53, 46). By isothermal distillation methods a value of 3,100 was found; this value agrees well with that obtained by chemical analysis of crystalline flavianate (3,000), assuming one molecule of flavianic acid per molecule of gramicidin (53). However, cryoscopic determinations in camphor gave values of 1,340 to 1,622 (32); when cyclohexanol was used the values were as low as 600 to 1,200 (53). These low values indicate that gramicidin may dissociate reversibly under certain conditions into two or four units of lower molecular weight.

Both gramicidin and tyrocidine react positively in most of the common protein reactions, such as the biuret, xanthoproteic, Millon's and Voisenet reactions. On hydrolysis a mixture of amino acids is obtained, most of which have been identified (see Table 1). Gramicidin appears to possess the simpler structure. The following building stones, in the order of their importance, have been definitely identified: *l*-tryptophane, *d*-leucin, *d*- and *l*-valine, *l*-alanine, and a 1-amino-2-hydroxy compound which is possibly iso-serine. Glycin may also be present (47). According to Gordon (47), the number of amino acid building blocks per molecule of gramicidin in the arrangement given is 6:6:5:3:2(:2). These five or six amino acids are arranged either as a large cyclic molecule or as an end-substituted peptide chain.

The hydrolysate of tyrocidine contains the following amino acids: *l*-tryptophane; tyrosin; alanine; phenylalanine; aspartic acid; a basic amino acid, not yet identified; and ammonia.

According to Lipmann *et al.* (52), about 45% of the amino acids of gramicidin and 20% of those of tyrocidine are present in the unnatural form. These observations have not been confirmed as yet under varied conditions of hydrolysis. If true it may form the basis for a satisfactory explanation for their antibiotic properties.

The most important chemical data of both gramicidin and tyrocidine are stated in Table 1.

Bacteriostatic and Bactericidal Properties of Gramicidin and Tyrocidine

Gramicidin undoubtedly is one of the most potent bacteriostatic agents known, equalled or only slightly surpassed in potency by pure penicillin. An amount of 0.01 to 0.02 mg. gramicidin per liter of broth is sufficient to inhibit the growth of most strains belonging to the species: *Diplococcus pneumoniae* (pneumococcus), *Strep. haemolyticus, Strep. faecalis, Strep. mastitides* and *Gaffkya tetragena* for a considerable period of time. Concentrations of 0.1 to 0.2 mg. gramicidin per liter of broth inhibits the growth of *Lactobacillus acidophilus, Staph. citreus* and *Mycobacterium phlei.* The most resistant Gram-positive species are *Staph. aureus, Bac. anthracis* and the sporulating anaerobes of the genus *Clostridium,* requiring 1 to 2 mg. of gramicidin per liter of broth. In order to prevent growth of all these species for an indefinite period of time, 10 to 20 times higher concentrations are required.

In general all species that are sensitive for gramicidin are also highly susceptible for tyrocidine. For the majority of susceptible species gramicidin is considerably more effective than tyrocidine. Usually five to ten times higher concentrations of tyrocidine are required to obtain the same inhibitory effect that gramicidin exerts. Interesting exceptions, however, are the spore-bearing species (*Bac. anthracis* as well as the gas-gangrene bacilli) where the ratio is almost the reverse. The same is true for meningococcus and gonococcus, two of the few Gram-negative species highly susceptible to both gramicidin and tyrocidine. Remarkably resistant to both gramicidin and tyrocidine are strains of *Staph. aureus.* Rammelkamp (38) observed that 12 of 19 recently isolated strains were permanently inhibited only by concentrations of tyrothricin ranging from 24 to 100 mg. per liter, and seven strains endured as much as 100 to 200 mg. per liter.

Undoubtedly the most outstanding property of gramicidin and tyrocidine, as well as of penicillin, is the almost perfect specificity

TABLE 1

	Gramicidin	Tyrocidine
Soluble in:	methanol, ethanol, dioxane, pyridin, glacial acetic acid and wet ethyl acetate, chloroform and acetone	
Insoluble in:	ether, petroleum-ether, benzole and carbon tetrachloride	
Chemical nature:	polypeptides, not digestible by proteolytic enzymes such as trypsin, papain, bacterial proteases, erepsin.	
Chemical analysis:	C: 62,7 % H: 7,6 % N: 14,8 % (Hoogerheide, 1940) (Hotchkiss-Dubos, 1941)	C: 59,6 % H: 6,7 % (–HCl-salt) N: 14,3 % Cl: 2,7 % (Hotchkiss-Dubos, 1941)
Molecular weight:	1340–1622 (in camphor) (Hoogerheide, 1940)	2700 (Christiansen *et al.*, 1941)
	3000 (flavianate analysis) (Tishler *et al.*, 1941)	$(2500)_n$ (Hotchkiss, 1943)
	3100 (isothermal distillation) (Tishler *et al.*, 1941)	
	600–1200 (in cyclohexanol) (Tishler *et al.*, 1941)	
	$(575)_n$ (Christiansen *et al.*, 1941)	
	$(1500)_n$ (Hotchkiss, 1943)	
Optical rotation $[\alpha_D]$	+5°	–105°

TABLE 1 (*Continued*)

	Gramicidin	Tyrocidine
Melting point:	228–232 (Hoogerheide, 1940) 228–230 (Hotchkiss-Dubos, 1940) 230–231 (Hotchkiss-Dubos, 1941)	230–234 (Hotchkiss-Dubos, 1940) 240 (Hotchkiss-Dubos, 1941)
Absorption spectrum:	Principle max. 2815 Å Secondary max. 2910 Å Minimum 2475 Å Extinction coeff. 12,6	
Positive protein react.:	Biuret, xanthoproteic (strong) Millon's (yellow color indicates tryptophane and no tyrosin) Voisenet (tryptophane)	
Negative protein reactions:	ninhydrin, Molisch	
Acid-base character:	neutral	weakly basic, with 2 amino groups and one carboxyl group
Amino acids accounted for:	*l*-tryptophane *d*-leucin *d*, *l*-valine *l*-alanine 1-amino-2-hydroxy-compound (iso-serine ?) (glycin)	*l*-tryptophane tyrosine alanine phenylalanine aspartic acid basic amino acid ammonia (dicarboxylic amino acid)

for Gram-positive bacteria. Whereas minute traces of these compounds inhibit the growth of practically all Gram-positive species, even large concentrations have no inhibitory effect whatsoever on the great majority of Gram-negative species. Although certain bactericidal dyes and detergents have tendencies in the same direction, none of the well known bacteriostatic or bactericidal chemicals such as phenols, mercurials, dyes or detergents exhibits a specificity for Gram-positive species in such extremes. A satisfactory explanation for this phenomena has never been given, but the close correlation between Gram-staining and tyrothricin activity is certainly not coincidental and is of importance enough to warrant a study of the problem. A few exceptions must be mentioned, however. We have already seen that a few Gram-negative species, such as gonococcus and meningococcus, are also susceptible to the action of both antibiotics. Moreover, several Gram-positive species, such as *Mycob. leprae, Mycob. ranae* and *Mycob. tuberculosum,* are only slightly susceptible to both gramicidin and tyrocidine.

The action of both components on Gram-positive bacteria is mainly bacteriostatic. However, higher concentrations, still relatively small, are decidedly bactericidal. When suspended in distilled water or a 5% glucose solution, a concentration as low as 0.1 mg. per liter of both agents will sterilize a pneumococcus suspension in 30 minutes at 37° C. For *Strep. haemolyticus* and *Corynebact. dipththeriae* (gravis), a concentration of 1 mg. per liter is required; for *Staph. aureus,* meningococci and gonococci 5 to 10 mg. per liter is necessary (42). In the absence of nutrient broth, using high concentrations of both antibiotics, several Gram-negative species, such as *Pasteurella avida, Past. tularensis, Shig. dysentheriae, E. coli, Salm. scottmuelleri* and *Eberth. typhosa,* are somewhat susceptible to the bactericidal action of gramicidin and especially tyrocidine. But even with tyrocidine, concentrations as high as 250 to 500 mg. per liter are necessary to obtain sterilization (13).

In the presence of broth, prepared from peptones, meat or yeast extracts, or meat infusion, the bactericidal activity of both antibiotics is greatly reduced. The Gram-negative species, with the exception of gonococci and meningococci, are not effected in the presence of broth, even not by tyrocidine. For Gram-positive species, about 10 to 50 times higher concentrations of both antibiotics are required to obtain sterilization in broth. Addition of blood, serum, milk or other protein-rich substrates to the medium has a tremendous effect on the bacteriostatic and bactericidal efficiency of both compounds, especially on tyrocidine. Practically all bactericidal activity is lost in the presence of blood and serum, and both antibiotics remain only bacteriostatic (41). In order to obtain the same inhibition of growth of pneumococci in the presence of 10% serum, about 60 times as much tyrocidine and 10 to 20 times as much gramicidin is required than in the absence of serum. Small amounts of serum also greatly reduce the hemolytic activity of tyrocidine; 5% serum completely prevents hemolysis (31).

It has been observed repeatedly that bacteria, which are highly sensitive to tyrothricin, may develop marked resistance to this substance when subjected for a considerable time to increasing low concentrations of the bactericidal substance. The same increase in tolerance has been observed with strains of *Staph. aureus* isolated from an ulcer of the leg, treated for a considerable period of time with tyrothricin.

The specific action of tyrothricin on Gram-positive bacteria can be utilized to facilitate the isolation of certain tyrothricin-resistant Gram-negative species from mixtures with Gram-positive species. Isolation of *H. influenzae* from the nasopharynx is facilitated by the use of a medium containing sufficient tyrothricin to prevent the growth of Gram-positive cocci (42). Isolation of *Neisseria gonorrhoeae* is also facilitated by a concentration of 1/15,000 of tyrothricin in "chocolate" agar, preventing the growth of diphtheroids, streptococci and *Lactob. acidophilus* but not of *Staph. aureus* and gonococci. Numerous other applications in this field are possible and undoubtedly will be made in due time.

The effect of tyrothricin as a growth inhibitor is not restricted to bacteria; growth of higher plants is also strongly inhibited by small amounts. Growth of corn roots, when submerged in a tyrothricin solution containing 10 mg. per liter, is considerably retarded, and a concentration of 60 mg. brings growth practically to a standstill, permanently injuring the roots. The effect seems to be due to the tyrocidine fraction. This observation is of some importance, for it is possible, in view of the general occurrence of *Bac. brevis* in soil, that tyrothricin is produced locally in soils.

Mechanism of the Bactericidal Action of Gramicidin and Tyrocidine

The mechanisms of inhibition by gramicidin and tyrocidine are fundamentally different. Tyrocidine causes complete inhibition of all metabolic functions of the cell. The oxidation-reduction systems, respiratory-enzyme system and glycolytic enzyme system as well as dehydrogenase activity of *Staph. aureus* and *Strep. hemolyticus* are strongly inhibited by concentrations of 40 mg. per liter. Its action leads to disintegration of the cell and even lysis (19, 28). Some bacterial species are very easily lysed by tyrocidine; others, however, are not susceptible to lysis. A five-hour-old broth culture of *Micrococcus conglomeratus,* for instance, is completely lysed upon addition of 4mg. tyrocidine per liter (44). Gramicidin, on the other hand, only partially injures the cells. Cell metabolism may be either inhibited or stimulated, depending on the conditions, such as presence of K^+ ions or Na^+ ions. Synthesis of adenosine triphosphate is inhibited, and its bacteriostatic action may be due partially to such injury. Undoubtedly more research is required to give a clear picture of the mechanism of inhibition by both antibiotic substances.

Toxicity

Both gramicidin and tyrocidine are highly toxic for laboratory animals when injected intravenously, intraperitoneally or subcutaneously. However, given orally, both are non-toxic, even in large dose administered at once or over a long period. Apparently no absorption from the intestinal tract occurs and both agents are probably totally immobilized. Daily feeding of mice with 2 mg. of tyrothricin failed to cause any appreciable reduction of the *L. acidophilus* flora in the faeces, although this species is very susceptible *in vitro* to tyrothricin (45).

Tyrocidine, nothwithstanding its powerful hemolytic action, is considerably less toxic than gramicidin. The approximate lethal doses for 20-gram mice are:

intravenous:	tyrothricin	0.1 mg.
	tyrocidine	0.5 mg.
	gramicidin	0.075 mg.
intraperitoneal:	tyrothrycin	1.6 mg.
	tyrocidine	1.8 mg.
	gramicidin	0.6 mg.

Death is generally due to respiratory failure, occurring within 24 hours after injection of gramicidin or in three to four days with tyrocidine. Dogs, injected daily for ten days with small amounts of tyrothricin (0.2 mg. per kg.), showed only minor evidence of toxicity. However, a double dose caused death, usually before the ten injections were completed. Three-tenths of a milligram per kg. was not fatal but caused well marked acute or chronic changes in the liver, spleen, kidneys, heart and lungs.

Notwithstanding the high toxicity on parenteral administration, use of tyrothricin on open wounds has been found to be quite safe. Apparently very little absorption occurs. Even when injected into closed cavities, such as the bladder, the pleural cavity and the udder of cows, no toxic effects are observed. For instance, a single injection of 200 mg. of tyrothricin into an infected pleural cavity of a human had no adverse effects (75).

Therapeutic Value

The extreme specificity of these compounds, exhibited against one type of living cell (Gram-positive bacteria) and not against another type (Gram-negative species), has drawn special attention to these compounds as possible therapeutic agents. Already Paul Ehrlich visualized that there might exist such specific chemical agents which would destroy pathogenic bacteria without a pronounced toxic effect on the cells of the host. However, besides this specificity, other factors also have to be taken in consideration. Briefly summarized, an ideal chemotherapeutic agent should possess the following properties:

a) high bactericidal or at least bacteriostatic properties for pathogenic bacteria;
b) body fluids, such as blood serum, exudate, should not inhibit this bactericidal action;
c) marked diffusibility in body fluids; therefore water solubility and of relative low molecular weight;
d) therapeutic ratio, the latitude between effectiveness against pathogenic bacteria and toxic effect on the host, as great as possible.

When we consider gramicidin and tyrocidine with these four criteria in view, it can readily be seen that both are remote from being ideal chemotherapeutic agents. Undoubtedly both are among the most potent bacteriostatic agents known. However, as was pointed out already, serum and blood greatly reduce their bacteriostatic effectiveness. Both are practically water-insoluble; only colloidal solutions can be prepared; electrolytes cause complete precipitation; and diffusibility is extremely poor. Finally, the toxicity of both compounds is considerable.

It is evident, therefore, that both substances have been found of no value whatsoever for treatment of infectious diseases, such as pneumonia and generalized streptococcal and staphylococcal infections. There are numerous reports claiming that gramicidin is active *in vivo* and exhibits complete protection against many lethal doses of pneumococci, streptococci, staphylococci, *Bac. anthracis* and other pathogens susceptible to the action of this agent *in vitro*. These reports, although essentially correct, are misleading in so far as the evaluation of the therapeutic value of these compounds is concerned. Protection is afforded only when the infective dose and the protective dose are injected a short time apart and at the same site, *e.g.*, both intraperitoneally, thus giving a direct contact of the germicide with the infective organisms before the latter have had an opportunity to invade their host.

As soon as infective and protective injections are given on different sites and the bloodstream has to transport the antibiotic agent to the site of infection, both gramicidin and tyrocidine are entirely ineffective, even in doses approaching the sublethal range.

However, for topical application and local use, as in the treatment of infected wounds, especially when not much exudate is present, both gramicidin and tyrocidine have proved to be of great value. One of the first practical applications of tyrothricin was in the field of veterinary medicine. Suspensions of it are used quite extensively for local treatment of bovine mastitis, a common disease in dairy cattle, usually caused by *Strep. agalactiae*. This organism is resistant to most of the sulfa drugs but very sensitive to tyrothricin. Considerable literature has accumulated on the treatment of this disease with tyrothricin, and there seems to be general agreement that it is one of the least irritating agents of several chemotherapeutic agents commonly used for treatment of this disease. Moreover, tyrothricin has been shown to give the highest percentage of recoveries, compared with other drugs. Often 80% to 90% of all cases treated are cured, usually with one treatment especially if the treatment is given when the quarters are dry (non-lactating cows). The solutions of tyrothricin, either in distilled water or as a water-mineral oil emulsion, are instilled into the udder *via* the teat-canal and left there for 12 to 18 hours. About 25 ml., containing from 30 to 150 mg. tyrothricin per quarter, gave the best results.

On humans tyrothricin is used very successfully for the treatment of infected ulcers, abscesses, sinusitis, osteomyelitis, empyema and suppurative wounds. It should be borne in mind, however, that good surgery should always be combined with application of tyrothricin, since this agent must be able to come in direct contact with the infecting organisms; one cannot depend on possible diffusion through tissue, exudate, pus or debris. Tyrothricin is most effective in dealing with streptococcal infections and considerably less effective in staphylococcal infections. This, of course, could be expected from *in vitro* results. For wound disinfection tyrothricin solutions are very satisfactory and cause no irritation or toxic effects. It is surprising that its use for this purpose has not been more extensive, especially for prevention of infection, including war wounds. Tyrothricin apparently is just as satisfactory for this purpose as gramicidin, and for therapeutic use there is no need to separate tyrothricin into its two fractions. The concentrations used for wound treatment vary considerably with the investigator. For large wounds as well as for treatment of empyema, 100 mg. of tyrothricin per liter of distilled water are sufficient. For smaller areas, however, higher concentrations can be used safely. For instance, for treatment of ulcers concentrations as high as five grams per liter have been used. Since it is impossible to prepare stable colloidal solutions of such high concentrations in water, alcoholic solutions or tinctures must be used. Solutions for wound treatment are either used in the form of wet dressings or directly sprayed on the wounds.

ANTIBIOTIC AGENTS EXCRETED BY ACTINOMYCES SPECIES

Considerable work has been done on the isolation of antibiotic agents excreted by species of *Actinomyces*. The name of Dr. S. A. Waksman is intimately connected with practically all research done on this type of antibiotics. This is hardly surprising, since this investigator is generally recognized as the most outstanding expert on this particular genus of micro-organisms. A large number of antagonistic Actinomycetes have been isolated by him and his co-workers, and a number of strains have been investigated more thoroughly. Some of the antibiotic agents have been isolated in chemically pure form; others have been highly purified. The most important are actinomycin A, actinomycin B, actinomycetin and streptothricin.

Actinomycin A and B

Waksman and Woodruff (83) isolated from soil in 1940 a new chromogenic species of *Actinomyces* which showed strong antagonistic properties toward all bacteria belonging to both the Gram-positive and Gram-negative types. This new species was later described as *Act. antibioticus.*

The antibiotic agent responsible for this inhibition is excreted on a large variety of media, varying from media rich in protein or peptones to pure synthetic media with starch as the source of carbon and a nitrate or, better, phenylalanine as the only source of nitrogen. Small amounts of agar are added to these media in order to prevent the delicate surface membrane from being submerged. The active agent can be extracted with ether from six- to eight-day-old cultures of this organism on nutrient media. Upon evaporation of the ether extract a crude powder is obtained which was called "actinomycin". By means of petroleum-ether, actinomycin can be separated into two crystalline fractions. Actinomycin A is insoluble in petroleum ether but limitedly soluble in water, and is highly pigmented (red). Actinomycin B, on the other hand, is soluble in petroleum ether but not in water, and is not pigmented. Fraction B is formed mainly during the early stages of growth, whereas fraction A is produced principally in older cultures.

Actinomycin A is the more active fraction and has been studied most extensively. One-one-hundredth of a microgram per ml. of broth is sufficient to inhibit the growth of several Gram-positive species, such as streptococci, staphylococci and *Bac. subtilis.* This activity is of the same order as that of gramicidin and penicillin. Other Gram-positive species, however, require as much as 10 micrograms per ml. (*Mycobact. tuberculosum, Bac. macerans*). Gram-negative species are also inhibited, although usually concentrations of 10 to 20 micrograms per ml. are required, even 100 micrograms for the coli-aerogenes group and *Serratia marcescens.* Generally speaking, Gram-negative species are more resistant to actinomycin

A than Gram-positive forms, but the sharp demarcation line between the two groups in regard to their sensitivity to gramicidin or penicillin does not exist for actinomycin A. There are marked differences in sensitivity for this antibiotic within the species of each group.

The extreme inhibitory action of actinomycin A is mainly of a bacteriostatic nature, similar to the action of gramicidin. Like the latter, only when much higher concentrations are employed, does bactericidal action become evident; *e.g.*, 10 to 50 micrograms per ml. are required for certain sensitive Gram-positive species. Actinomycin B, on the other hand, lacks the bacteriostatic range which precedes bactericidal action. Its effect is mainly bactericidal, and in this regard it resembles tyrocidin. The bactericidal potency of actinomycin A and B is of the same order.

Actinomycin is also quite fungicidal, especially against fungi of the genus *Penicillium*. It is also quite active against several actinomycetes.

Actinomycin A is a crystalline substance of a melting point of 250° C. and is optically active $[\alpha_D^{25}] = -310 \pm 5$. It has a molecular weight of about 800 and the following composition: C—59.0%; H—6.8%; N—13.4%; O—20.8%. It is a polycyclic N-compound, the exact configuration not being known. Several groups can be acetylated, and it is a reversible oxidation-reduction system, apparently of the quinone type. In this regard it resembles chlororaphin, the antibiotic pigment of *Pseudomonas chlororaphis*. Actinomycin A is readily soluble in chloroform, benzene and ethanol, moderately soluble in acetone and hot ethyl acetate, and sparingly soluble in water and ether. It is quite stable; a neutral solution can be boiled for 30 minutes without loss of activity; however, in acid or alkaline solution it is slowly decomposed. From among 250 strains of actinomycetes tested it was found to occur only in strains of *Act. antibioticus*.

Since actinomycin A is sufficiently soluble in water, highly bacteriostatic and not inhibited in its action by the presence of serum or other body fluids, it has most of the properties required of a good chemotherapeutic agent. Unfortunately, however, it is extremely toxic, even in oral administration. Its L.D.-50 (dose lethal for 50% of the animals tested) is as low as 0.35 mg. per kg. for mice, compared with 30 mg. for gramicidin and 90 mg. for tyrocidine.

Death apparently is due to respiratory failure and usually does not occur until 15 to 20 hours after administration. Even such traces as 50 micrograms per kg., given intraperitoneally, produce death in mice or rats when administered daily for six days. Gross pathological changes can be observed, such as marked shrinkage of the spleen. Liver and kidney functions are also affected. Actinomycin A is rapidly removed from the blood, partly excreted in the urine and partly stored in many organs of the body.

Due to its extreme toxicity, actinomycin A has no therapeutic possibilities, not even for topical application on open wounds.

Active actinomycin-containing filtrates can be produced by growing *Act. antibioticus* in flasks in shallow layers of tryptone-glucose broth. The composition of the medium as well as the technique for obtaining good surface growth will be outlined in more detail in the discussion of streptothricin. Actinomycin filtrates of considerably higher titer can be produced by the submerged culture technique. This technique is becoming increasingly important in research on as well as production of antibiotics, especially penicillin, streptothricin, actinomycin and tyrothricin. It consists of growing strictly aerobic species of bacteria, actinomycetes or fungi under submerged conditions and supplying the culture with a generous amount of sterile air, either by continuous vigorous agitation in flasks or by passing an uninterrupted stream of sterile air through the nutrient liquid. Often it is necessary to combine this aeration with vigorous stirring to disperse the air bubbles. Growth of actinomycetes and fungi under such conditions is entirely different from the pellicle or matt observed in stationary cultures. Discrete colonies and mycelial fragments are produced throughout the liquid. Growth is much more rapid and abundant and of surprising uniformity. Consequently results obtained by the submerged culture technique are more reproducible, and usually higher titers of the antibiotics are obtained in a shorter period of time. Moreover, this technique is ideally adaptable to large-scale production of antibiotics in tanks equipped with aeration devices and provided with mechanical stirrers. For laboratory research, 500-ml. Erlenmeyer flasks with 150 to 200 ml. of inoculated media, continuously shaken by a shaking-machine in a constant temperature room, are very well adapted for submerged culture study of actinomycetes and fungi. The importance of this newly developed technique for the study of antibiotics can not be stressed too much.

As far as actinomycin production is concerned, filtrates may be obtained after an incubation period of one week, inhibiting the growth of *Bac. subtilis* in a dilution of 1/200 for stationary cultures or 1/400 for submerged cultures. Actinomycin A is not produced until maximum growth has been reached, usually not before the fifth day. It is, therefore, entirely possible that it is an autolytic product, liberated after active growth has ceased.

Actinomycetin

Strictly interpreted, actinomycetin is not an antibiotic agent. However, it is usually discussed with antibiotics and therefore will be considered here briefly. In 1936 it was observed by Welsch that during sporulation a large number of *Actinomyces*-strains (about 50% of all strains tested) liberate a bacteriolytic agent into the medium. This agent causes lysis of a large variety of heat-killed Gram-positive and Gram-negative species. This bacteriolytic principle (actinomycetin) is produced only in older cultures of *Actinomyces* on a large variety of media supporting good growth of these organisms. Broth of neutral or slightly alkaline pH distributed over flasks in shallow layers, gives the highest yields. The lytic action of crude actinomycetin is especially pronounced on suspensions of heat-killed Gram-negative bacteria. Clarification is achieved in a few hours. Heat-killed Gram-positive bacteria are also lysed, but at a much slower rate; usually 24 to 48 hours incubation is required.

Living streptococci and pneumococci may also be lysed, but only by filtrates of a restricted number of *Actinomyces* strains. There are indications that these strains excrete weakly bacteriostatic or bactericidal agents which may destroy the living cells before they become susceptible to the lytic action of actinomycetin. For instance, by extraction with ether of certain crude actinomycetin preparations, Welsch isolated a fraction from the ether phase which inhibited the growth of *Staph. aureus*, pneumococci, streptococci and several species of the genus *Bacillus* in concentrations of 0.2 mg. per ml. However, very little attention has been paid to these bactericidal fractions, and it is entirely possible that the combined action of such bactericidal impurities with actinomycetin is the reason for the lysis of living bacteria.

Actinomycetin (the bacteriolytic fraction) is a protein, soluble in water, glycerol and dilute saline, and is precipitated by alcohol, acetone or ammonium sulphate. It is heat labile, being inactivated by exposure to temperatures of 60° to 70° C. It is also inactivated by ultra-violet light of a wave length shorter than 300 mμ.

Streptothricin

The name "streptothricin" is derived from the early generic designation, *Streptothrix,* given to the group of organisms now known as *Actinomyces.* It is one of the latest additions to the growing list of antibiotics, and there are indications that this substance may become an important chemotherapeutic agent. There is relatively little information available about it, but several laboratories are doing considerable research on the substance.

Streptothricin was first isolated as a crude extract by Waksman and Woodruff (87) from culture filtrates of a strain of *Actinomyces* isolated from soil and later identified as *Act. lavendulae.* It was found that different strains of this species vary widely in their ability to produce streptothricin. It is essential, therefore, that a very active strain be used for streptothricin production.

Production of active streptothricin filtrates. The following medium gives good yields of streptothricin (88, 89):

$$\left.\begin{array}{l} 1\% \text{ starch (or glucose)} \\ 0.5\% \text{ tryptone} \\ 0.05\text{–}0.2\% \ K_2HPO_4 \\ 0.05\text{–}0.2\% \ NaCl \\ 0.01\% \ FeSO_4 \end{array}\right\} \text{per liter of tap water.}$$

Amounts of 200 ml. or less (pH may vary between 5.0 and 7.5) are distributed over one-liter Erlenmeyer flasks and sterilized for 15 minutes at 10 lbs. pressure (glucose should be sterilized separately). A shallow layer of medium, not more than one inch thick, is essential for good production; the shallower the layer the higher the ultimate titer. Each flask is inoculated with spores of *Act. lavendulae* from a slant or agar plate, care being taken that the spores remain afloat on the surface of the medium. This can usually be achieved by mixing the spores with sterile talcum powder and using about 100 mg. of the mixture per flask. The spores on the surface develop into a dry entire pellicle which should not be disturbed. Spores that become submerged develop into submerged colonies which do not produce streptothricin. In order to prevent the pellicle from falling to the bottom, ¼% agar may be added to the medium, or absorbent cotton packed up to the surface of the liquid may be used. When starch is employed in the medium and talcum powder on the surface, further precautions are usually unnecessary. Good streptothricin production depends largely on good growth and good growth is correlated with maintenance of a dry pellicle.

The inoculated flasks are incubated for 10 to 14 days at 20° to 25° C. At higher temperatures (maximum 30° C.) streptothricin is produced in a shorter time but the ultimate titer is considerably lower. There is a definite optimum incubation period; beyond this the streptothricin yields decrease rapidly. A dilution of 1/200 to 1/500 of a good active filtrate of *Act. lavendulae* is capable of inhibiting the growth of *Bac. subtilus,* and a dilution of 1/50 to 1/100 will prevent growth of *E. coli.*

Glutamic acid or glycin may be substituted for the tryptone in the medium with comparable results.

A second method of producing active filtrates of *Act. lavendulae* is by the submerged culture technique, either in flasks shaken continuously by mechanical means or in vessels provided with stirring devices, by passing sterile air through the medium. Both procedures lead to abundant submerged growth in a very much shorter time period than under corresponding stationary surface conditions. Streptothricin production under submerged conditions is not subjected to such extreme variations as sometimes are encountered in surface cultures; moreover, considerably higher titers are obtained. Maximum activity is usually reached in five to seven days and coincides with maximum growth. Substitution of 3% neutralized corn steep liquor or 0.6% soybean meal for tryptone in the medium increases the titer considerably.

Isolation of streptothricin. The active filtrates are collected, filtered, and shaken with Norite A. The charcoal which absorbs all activity from the filtrate is collected on a filter and extracted with acidified alcohol. After neutralization of the extract with NaOH, ten volumes of ether are added. A highly concentrated aqueous solution of streptothricin separates. It may be concentrated further by evaporation at reduced pressure. Although streptothricin has not been crystallized, highly purified preparations have been prepared, but not much information on the technique for purification is available. Streptothricin is a nitrogen-containing base, insoluble in ether, petrolether and chloroform, but soluble in water.

Characteristics of Actinomyces lavendulae. During growth in complex nitrogenous media, both as shake culture and as stationary culture, there is rapid utilization of organic nitrogen compounds, far in excess of the requirements for cell synthesis. Considerable deamination occurs and ammonia is liberated. Carbohydrates are also vigorously attacked and acids are produced, largely lactic acid. During the early stages of growth, acid production predominates over deamination. Consequently, a drop in pH is observed from 7.0 to about 6.0, followed by a steady rise during the later stages of growth. Maximum streptothricin titers are reached as soon as this rise in pH becomes apparent. The drop in pH can and should be largely controlled by choosing the proper ratio of carbohydrate and protein. When this ratio is too great, a sharp drop in pH is observed, sometimes as low as 3.2; when it is too low, the medium becomes very alkaline, due to accumulation of ammonia.

Characteristics of streptothricin. Streptothricin is unique as far as its bacteriostatic specificity is concerned. Gram-negative and Gram-positive species are equally susceptible to its action. However, in both groups there are species that are highly resistant, and others that are highly susceptible. For instance, among the Gram-positive species, *Bac. subtilis* is very sensitive, but a genetically closely related species, *Bac. mycoides,* is resistant. Among the Gram-negative species, *E. coli, Brucella abortus* and several species of *Shigella* are very sensitive, but *Ps. fluorescens* is resistant.

Streptothricin is bactericidal as well as bacteriostatic. It is quite soluble in water, relatively stable and heat-resistant; it is active in the presence of serum and has low toxicity for animals. These properties, of course, are of particular importance for possible therapeutic application. However, only *in vivo* experiments of a preliminary nature have been reported, and its therapeutic possibilities have not been fully explored.

ANTIBIOTIC SUBSTANCES FROM OTHER SOIL BACTERIA

It has been pointed out already that there is an unlimited number of bacteria in the soil that can excrete bactericidal substances under proper conditions. An extensive field for valuable research is here awaiting exploration, and it is possible that valuable chemotherapeutic agents may be discovered. Unfortunately, little attention has been paid to it, and the promising results reported in the literature have never been carried further. Although a large number of antibiotic substances derived from soil bacteria have been discovered, only very few of them have been isolated, purified, identified and tested for possible therapeutic value. The most important are pyocyanase and pyocyanin.

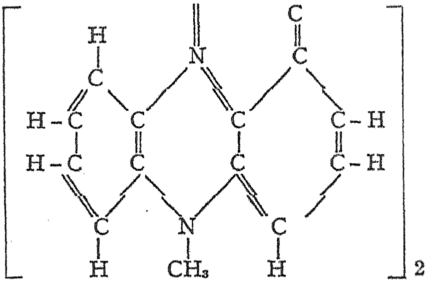
Both these substances are excretion products of certain strains of *Pseudomonas aeruginosa.* Undoubtedly this was the first important antagonist to receive attention. In general, excretion of antibiotic substances by soil bacteria as well as by molds is highly "strain-specific" and not "species-specific". Often a considerable decrease or even loss of the ability to excrete such agents may be observed on subculturing. This is especially true of *Ps. aeruginosa.* Some strains produce only pyocyanase, others produce both pyocyanase and pyocyanin, whereas still others produce no active agent at all.

According to Goreczky (108), excretion of pyocyanase is closely paralleled by the virulence of the strain. It is produced only on complex nitrogenous media and not in synthetic media. Glycerol-bouillon apparently gives optimal yields. It may be prepared by extraction of a culture medium of a suitable strain with ether or petroleum-ether. This crude extract consists mainly of a neutral fat, a phosphatide, both inactive, and a mixture of fatty acids of high molecular weight possessing hemolytic, bacteriostatic and weakly bactericidal properties. The unsaturated fatty acids are the most active, their activities being proportional to their number of double bonds.

Pyocyanase is active against a large variety of Gram-positive as well as Gram-negative species including *Bac. anthracis, Corynebacterium diphtheriae, Strep. haemolyticus, Staph. aureus* and *albus, E. coli, Eberth. typhosus, B. dysenteriae* and *Brucella abortus.* It is highly haemolytic, however, as well as surface active. Its toxicity for animals is relatively low. For a method of preparation in reasonably pure form, see 113 and 116 in the bibliography.

Pyocyanin is a blue-green pigment elaborated by many strains of *Ps. aeruginosa* on complex nitrogenous media as well as on pure synthetic media with NH_4-lactate as the source of nitrogen and carbon. It can be obtained in good yield by growing the organism for 10 to 14 days at 25° C. in very shallow layers of a medium containing 1% peptone, ½% NaCl and 12% gelatin. It may be extracted from the slightly alkaline culture media with chloroform in which it is soluble with a dark blue color. From the chloroform phase it can be taken up in small amounts of dilute HCl in which it is soluble with a deep red color. After addition of bicarbonate it can be taken up again in chloroform, and after thorough drying of the chloroform solution with water-free Na_2SO_4, it is precipitated as the crystalline HCl-salt by exposing the chloroform solution to HCl vapors.

Pyocyanin is a methyl derivative of α-hydroxy-phenazine. It has been synthesized by Wrede from this compound by treatment with dimethylsulphate (125–129). Its structure is:



Pyocyanin inhibits the growth of numerous species of bacteria, Gram-positive as well as Gram-negative, yeasts and fungi. Its activity in general exceeds that of pyocyanase; it is not excessively toxic; and in small concentrations it markedly enhances the respiration of many bacteria species as well as tissue cells.

Other bacterial pigments belonging to the phenazine-group. The yellowish-green pigment produced by *Pseudomonas chlororaphis* (chlororaphine) as well as that produced by *Chromobacterium iodinum* are related to pyocyanin and have been chemically identified. Chlororaphine is a quinhydrone-like complex of equimolar proportions of phenazine-*l*-carboxamide and dihydrophenazine-*l*-carboxamide; the pigment produced by *Chrom. iodinum* is probably NN′-dioxide of 1,2-dihydroxy phenazine. The latter pigment is quite active against *Strep. haemolyticus* and other Gram-positive organisms. An amount of 2 mg. per liter prevents growth of these organisms. This concentration is of the same order as for pyocyanin but about a hundred times as much as required for the more potent antibiotics, gramicidin, actinomycin or gliotoxin.

In general, it is very difficult to compare the activity of different antibiotics investigated by different investigators from the data available in the literature. It would be highly advisable, therefore, that a comparative study be made of the bacteriostatic and bactericidal properties of pure pyocyanase, pyocyanin, chlororaphin, the pigment of *Chrom. iodinum* and of many other antibiotics, under strictly comparable conditions against a number of the common pathogens in the presence and absence of serum. The 50% lethal dose and the possible therapeutic values of these compounds should also be determined.

Antibiotic agent from Pseudomonas fluorescens. Lewis (134) observed that a strain of *Ps. fluorescens* produces a thermostable, dialyzable agent, bacteriostatic and bactericidal for a large number of bacteria species, especially those of the genera *Bacillus* and *Micrococcus*. Representatives of the coli group, however, were resistant. The active principle is soluble in alcohol and adsorbed by charcoal, but has not been studied further.

Antibiotic agent from Serratia marcescens. Eisler and Jacobsohn (131) found that sterile broth cultures of a particular strain of *Serratia marcescens* contained a thermostable substance which inhibited the growth of *Corynebacterium diphtheriae,* gonococci and other species. No further work has been done with the active principle.

Antibiotic agents from representatives of the genus Bacillus. Undoubtedly the highest percentage of antagonistic strains of soil bacteria is found in the genus *Bacillus.* The excretion products of *Bac. brevis* are the only antibiotics of this group that have been investigated thoroughly. However, many related species excrete similar antibiotics which are not identical with gramicidin or tyrocidine. The fact that antibiotic products can be isolated by using the same procedure, as for tyrothricin, made certain investigators conclude that the active agent is tyrothricin. Usually such a conclusion is not permissible, even when the "bacterial spectrum" is similar to that of tyrothricin. Referent found on at least two occasions that the absorption spectrum in ultra-violet light of two such agents, isolated from two unidentified *Bacillus*-strains, was entirely different from that of gramicidin and tyrocidine, proving that both are chemically different compounds.

As early as 1940 Referent pointed out that certain strains of the most common spore-formers, among which was *Bac. subtilus,* excrete bactericidal products during growth in broth. Small amounts of crude active material were isolated by methods similar to those described for tyrothricin, but none of these crude active fractions was further investigated. Katznelson (1942) and Himfeld and Feustel (1943) confirmed the excretion of antibiotic substances by strains of *Bac. subtilis.* The active filtrates were found to be heat stable and effective against *Micrococcus conglomeratus* and *Staph. aureus.* The active principle has not been isolated and purified as yet, and nothing is known of its activity *in vivo.*

Water-soluble, highly active, bactericidal agents were also found in cultures of *Bac. vulgatus,* and an unidentified yellow spore-bearing bacillus (130). These agents were effective against various bacteria and fungi, including *Staph. aureus, Strep. lactis, E. coli, Eberth. typhosus* and *Lactob. acidophilus.*

Stokes and Woodward (135) isolated highly potent extracts from several antibiotic soil organisms, including *Bac. mesentericus* and *Bac. adhaerens,* which were active against various Gram-positive bacteria, but no further work has since been published on these agents.

Antibiotic substances against plant pathogens. An extensive literature has accumulated on this subject, indicating that antibiotic substances excreted by certain species of soil bacteria may be active in the natural control of soil-borne plant diseases of bacterial or fungal origin. None of such antibiotic substances has as yet been isolated chemically pure or even as a crude extract. Literature on strains of molds and soil bacteria, antagonistic to plant pathogens such as different species of *Fusarium, Ustilago zeae, Helminthosporium sativum, Ophiobolus graminis* and *Phymatotrichum omnivorum,* has been extensively reviewed (145). A great variety of antagonistic strains of molds and soil bacteria, many of them unidentified, have been isolated and found to excrete antibiotic substances against plant pathogens. Certain strains of *Bac. simplex* and *Bac. mesentericus* and some species of *Pseudomonas* and *Achromobacter* are a few examples.

Seeding of soil with such antagonistic strains or spraying with culture filtrates of them was found in some cases to check the prevalence of certain plant pathogens and to prevent disease. No systematic study of this subject has been made. The practical significance of prevention or cure of plant diseases by use of antibiotic substances should not be underestimated, and a thorough study of this subject seems more than justified.

ISOLATION OF ANTAGONISTIC BACTERIA SPECIES FROM SOIL

The following procedure has been used by Referent very successfully for the isolation of a large number of soil bacteria, excreting antibiotic substances.

A sterile 1% peptone-agar medium is inoculated just before solidification with a few drops of a suspension of *Mycobact. phlei* in sterile distilled water. This organism is quite susceptible to antibiotic substances and is slow growing. Any other organism that possesses a combination of these two properties can also be used. After pouring a number of plates with this inoculated agar, each plate is streaked with one drop of a dilute soil suspension. The dilution of the soil suspension should be such that a large number of separate colonies is produced without too much crowding. A few drops of glycerin on a small piece of filter paper placed in the cover of the Petri dish, will avoid "spreading" of certain types of colonies. The plates are incubated at 30° to 35° C. for two days. Several colonies on the agar surface may then be found surrounded by a more or less pronounced sterile halo, indicating excretion of bactericidal substances against *Mycobact. phlei.* Colonies surrounded by a sterile halo are isolated and usually prove to be equally active against a large variety of other species. The principle of this technique is to suppress the growth of the test organism (*Mycobact. phlei*) sufficiently long in order to allow time for the "antagonist" to develop into a colony and excrete sufficient antibiotic substances before the test organism develops.

A second method successfully used by Referent for obtaining antagonistic strains was similar to that just described, but sterile agar was used. As soon as the colonies of the soil bacteria had developed (usually after 24 hours incubation) the remaining spaces between the colonies were inoculated with *Bac. mycoides.* This organism forms colonies that gradually cover the total surface of the plate. However, it avoids those colonies that have excreted bactericidal substances.

The great majority of antagonistic species isolated by both methods belonged to the genus *Bacillus.* However, also antagonistic strains of *Pseudomonas* and *Actinomyces* were obtained.

If representatives of the genus *Bacillus* are desired it is better to pasteurize the soil suspension before streaking it on nutrient agar. For isolation of *Pseudomonas* antagonists, surface water is used instead of soil suspensions, and for *Actinomyces* antagonists, compost suspensions or other material naturally rich in such species.

As has been pointed out before, a whole field for research is wide open here. The simple technique for isolation of antagonists given above makes an abundance of antagonistic strains available for study to every bacteriologist interested in the subject. Although the soil microbiologist has a professional advantage and is more familiar with bacterial soil population than the average bacteriologist, there is no need for the latter to wait until supplied with antagonistic strains. However, collaboration with a chemist is often advisable, especially for isolation, purification and identification of the active agents. Isolation and description of antagonistic strains and evaluation of crude filtrates is of little value, since the involved data often are available in the bacteriological literature of half a century ago. Attempts should always be made to isolate, purify and identify the active agent. Its "bacterial spectrum" should be determined in the presence and absence of serum, its toxicity for laboratory animals, and finally its therapeutic value in experimental infections, avoiding direct contact between the infective organism and the therapeutic agent. Investigations of "crude extracts" should be limited, especially when it is possible.to obtain purer products by further study. Often those crude fractions are mixtures of two antibiotics and give confusing and misleading results.

Finally, a few words on the nomenclature of new antibiotics. The most logical designation undoubtedly is a name related to that of the species from which an antibiotic is obtained and not one related to the genus. Names such as penicillin and actinomycin may become misleading as soon as similar bactericidal agents are isolated from other species of the same genera.

SUMMARY AND CONCLUSIONS

The isolation of *Bacillus brevis* by Dubos in 1939 and the subsequent study of the active principles excreted by this species, have opened a new field for discovery of new chemotherapeutic agents for human, animal and possibly also for plant diseases.

Gramicidin and Tyrocidine. Both these agents have been isolated from cultures of *Bac. brevis.* This organism is a common inhabitant of the soil and may be isolated by a special technique. Both agents are polypeptides and have been obtained in crystalline form, chemically pure. Although the amino acid building components are known for both substances, their chemical configurations can not be determined with our present knowledge of protein chemistry. Both agents act almost exclusively on Gram-positive bacteria, especially on pneumococci and streptococci. This extreme specificity is quite unique. Moreover, both agents represent the most potent bacteriostatic substances known. Serum inhibits their action greatly. Since both are quite toxic, they are not suitable for internal use as chemotherapeutic agents. For local treatment of infected wounds, ulcers, *etc.* they have been found to be of great value.

Actinomycin A and B. Both these substances are isolated from cultures of *Actinomyces antibioticus* and have been prepared in crystalline form. Actinomycin A, a red pigment, is the more active, comparable in activity with gramicidin. It acts mainly on Gram-positive species but lacks the extreme specificity of gramicidin. Higher concentrations of actinomycin A also inhibit Gram-negative species. This substance is a polycyclic N-compound, but the exact configuration is not known. Its extreme toxicity, even by oral administration, makes it unsuitable for therapeutic use. Actinomycin B is considerably less active and has not been studied very extensively.

Actinomycetin. This agent is excreted by a large number of *Actinomyces* species. It is a lytic agent and, strictly speaking, not an antibiotic agent, since for its lytic action mainly dead bacteria are necessary. Often, however, it is contaminated with unknown antibiotic agents.

Streptothricin. This agent is an excretion product of *Actinomyces lavendulae.* It does not exhibit specificity for Gram-positive species, but some of the latter as well as certain Gram-negative species are quite sensitive to it. It is water-soluble, active in the presence of serum and has low toxicity for animals. It may become an important chemotherapeutic agent.

Other antibiotic agents. Among the antibiotic agents, known long before interest in this field was wide-spread, are:

a) pyocyanin, the blue-green pigment of *Pseudomonas aeruginosa;* this substance is less active than either streptothricin, actinomycin or tyrothricin, but shows activity against a large variety of Gram-positive as well as Gram-negative species; it has been crystallized, its structure is known and it has been synthesized;

b) chlororaphin, the pigment of *Pseudomonas chlororaphis,* chemically related to pyocyanin but is less active;

c) a number isolated from cultures of *Pseudomonas fluorescens, Serratia marcescens, Bacillus subtilus* and other spore-formers; they have not been purified and studied sufficiently.

It is not so much the few antibiotic agents that have been isolated, partly or completely identified and studied, that comprise the main contribution of soil microbiology to chemotherapy. In fact, it is entirely possible that very few of the many agents excreted by antagonistic soil bacteria will prove to be of any value chemotherapeutically. The important feature is that after an antibiotic agent has been identified chemically, it becomes possible to prepare derivatives of it that may be far better than the mother substance produced by micro-organisms. In other words, *via* antagonistic soil bacteria and molds and isolation and identification of the antibiotic agents excreted by such antagonistic strains, the chemist is guided to a class of compounds among which valuable chemotherapeutic compounds may be hidden. Soil microbiology may even become the basis on which chemotherapy can develop from the almost complete empirical stage of today to a broad science of utmost importance tomorrow. Although very little attention so far has been paid to antibiotic substances against plant pathogens, including fungi, the broad principles outlined in this review are also adaptable for this group of pathogenic organisms. A valuable research field is waiting here for proper exploration.

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